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**Investigating epigenetic dysregulation of Nociceptin
opioid peptide receptor (NOP) in eating and weight
disorders**

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Widmung

„Man darf nie an die ganze Straße auf einmal denken, verstehst du? Man muss nur an den nächsten Schritt denken, an den nächsten Atemzug, an den nächsten Besenstrich. Und immer wieder nur an den nächsten.“ [...] Und abermals nach einer langen Pause fuhr er fort: „Auf einmal merkt man, dass man Schritt für Schritt die ganze Straße gemacht hat.“
– aus *Momo* von Michael Ende

Ich möchte diese Arbeit gerne all meinen
Wegbereitern und Wegbegleiter widmen.
Ganz besonders aber meiner Familie.
Vielen lieben Dank für eure Unterstützung bei jedem Schritt

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List of Abbreviationindex

Abbreviation	Expansion
AN	Anorexia Nervosa
BED	Binge Eating Disorder
BMI	Body Mass Index
BWL	Behavioral Weight Loss Therapy
CI	Confidence Interval
CpG	Cytosine-phosphate-Guanine dinucleotide
DNA	Deoxyribonucleic Acid
DSM-IV/DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 4th/5th Edition
Df	Degrees of Freedom
EDE-Q	Eating Disorder Examination Questionnaire
EDTA	Ethylenediaminetetraacetic acid (anticoagulant)
ICD-11	International Classification of Diseases, 11th revision
NB	Naltrexone-Bupropion
N/OFQ	Nociceptin/Orphanin FQ (peptide)
NOP	Nociceptin opioid peptide receptor
p	Probability value (p-value, statistical significance)
PCR	Polymerase Chain Reaction
SD	Standard Deviation
SE	Standard Error
T	Test statistic (T-test value)
WHO	World Health Organization

1 Introduction

The introduction provides background on the key topics of this thesis: binge eating disorder (BED), DNA methylation, and the Nociceptin Opioid Peptide Receptor (NOP). Building on existing literature, the development of the hypotheses first considered current knowledge on DNA methylation in eating disorders and obesity, explored sex-specific DNA methylation differences, and examined potential links between BMI and DNA methylation levels (Chapters 1.2.1–1.2.3). These insights suggest that DNA methylation may differ between individuals with BED and those with obesity, vary by sex, and correlate with BMI. Drawing on this evidence, the NOP gene was selected as the focus of investigation, providing a framework to test these hypotheses in a gene-specific context.

1.1 Binge eating Disorder

Eating disorders are complex mental health conditions characterised by severe and persistent disturbances in eating behaviours, thoughts, and emotions (Giel et al., 2022a). While these issues may manifest as concerns related to food, weight, or body image, their underlying causes frequently extend beyond these domains, often delving into more profound psychological and emotional issues (Treasure et al., 2020). These conditions manifest in individuals across a diverse range of gender, age, ethnic, and body size demographics, although they are most frequently diagnosed in adolescents and young adults. The condition, which includes anorexia nervosa (AN), bulimia nervosa (BN) and binge eating disorder, has been shown to cause significant emotional distress and to carry serious medical risks, impacting almost all organ systems in the body. A combination of genetic, psychological, and sociocultural factors contributes to their development, and effective treatment must address both the mental and physical aspects of the illness (Giel et al., 2022a). It is evident that eating disorders share numerous similarities. The present thesis will focus on BED, which differs particularly with regard to the phenomenon of weight gain. Within the spectrum of eating disorders, BED is frequently associated with overweight or obese body mass (Blomquist et al., 2011), whereas BN for the most part is not. Conversely, AN is characterised by underweight body mass.

The presence of emotional distress and loss of control is a fundamental aspect of BED, yet the absence of purging or extreme restriction is also a salient feature (Camacho-Barcia et al., 2024). The following section outlines the core diagnostic criteria for BED.

1.1.1 Diagnostic Criteria for BED

BED is a relatively new diagnostic category within the field of eating disorders. It was first included in the DSM-IV in 1994, but not as an acknowledged diagnosis; rather, it was included as a research criteria (Lock and Osipov, 2024). In order for a BED diagnosis to be assigned, the following criteria must be met. While this study refers to individuals diagnosed according to the criteria set out in the DSM-IV, it is important to note that the current edition, the DSM-5, has since updated the diagnostic thresholds and severity specifiers. Both versions are presented in Table 1 (American Psychiatric Association, 1994, American Psychiatric Association, 2013)

In order to be diagnosed with BED the individual must satisfy all five of the established criteria. In the absence of any of these elements, the diagnosis cannot be made in accordance with current standards. The severity grading (mild, moderate, severe, extreme) is applied only after the diagnosis is established, based on the number of binge episodes per week (American Psychiatric Association, 2013).

The ICD-11 criteria demonstrate a high degree of similarity to DSM-5, with the requirement for all essential features to be present, in addition to the stipulation that symptoms are not better explained by another diagnosis (Quadflieg et al., 2023).

Table 1 Diagnostic criteria and severity grading for BED as defined by DSM- IV and DSM-5 (American Psychiatric Association, 1994, American Psychiatric Association, 2013)

Criterion 1: Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following • Eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat in a similar period of time under similar circumstances • The sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating)
Criterion 2: Binge-eating episodes are associated with three (or more) of the following • Eating much more rapidly than normal • Eating until feeling uncomfortably full • Eating large amounts of food when not feeling physically hungry • Eating alone because of being embarrassed by how much one is eating • Feeling disgusted with oneself, depressed, or very guilty after overeating
Criterion 3: Marked distress regarding binge eating is present.
Criterion 4: The binge eating occurs, on average, • at least 2 days a week for 6 months (DSM-IV frequency and duration criteria) • at least 1 day a week for 3 months (DSM-5 frequency and duration criteria)
Criterion 5: The binge eating is not associated with the regular use of inappropriate compensatory behavior (e.g., purging, fasting, excessive exercise) and does not occur exclusively during the course of anorexia nervosa or bulimia nervosa.
Severity Grading The DSM-IV does not include a grading scale for BED severity. The following grading scale is applicable to DSM-5 only. ○ Mild: 1 to 3 episodes per week ○ Moderate: 4 to 7 episodes per week ○ Severe: 8 to 13 episodes per week ○ Extreme: 14 or more episodes per week

1.1.2 Epidemiology and aetiology of BED

BED is a significant public health concern with varying prevalence across different populations (Giel et al., 2022a). A survey conducted in 14 countries with 24,124 respondents (aged 18 and above) across 14 predominantly upper-middle and high-income countries evaluated the lifetime prevalence of BED at 1.9%, while the one-year prevalence was 0.8%. The German population was included in this study, demonstrating a lifetime prevalence of 0.5% and a 12-month prevalence of 0.1%. These rates increase with body weight, with the median age of affected individuals being 21 years old (Kessler et al., 2013). The majority of individuals diagnosed with eating disorders are women (Treasure et al., 2020). However, sex does not necessarily play a as dominant role in the development of BED. In fact, approximately 40% of individuals diagnosed with BED are men (Hudson et al., 2007, Kessler et al., 2013). Currently, it is estimated that 2% of men and 3% of women will experience BED at some point in their lives (Lindvall Dahlgren et al., 2017).

The aetiology of BED is understood as the resultant of a dynamic interplay between biological predispositions and psychosocial influences. BED has been observed to occur with a high degree of frequency within familial contexts (Fowler and Bulik, 1997, Javaras et al., 2008), regardless of the presence of a tendency towards obesity within these families (Javaras et al., 2008). Twin and family studies indicate moderate-to-high heritability between 0.39 and 0.57 (Javaras et al., 2008, Mitchell et al., 2010, Thornton et al., 2011) and the genetic liability for BED overlaps not only with anthropometric traits such as overweight and obesity but also with broader psychiatric vulnerability, including risk alleles linked to schizophrenia, depression, and ADHD (Hübel et al., 2019a). The interaction of these numerous factors which include genetic, neurobiological, cognitive and socio-environmental elements may result in a positive feedback loop that could lead to the development of BED. One potential cause of excessive eating in the context of BED is the dysregulation of food intake signalling pathways. These interactions are

complex and involve the gastrointestinal, endocrine, and nervous systems, integrating metabolic, hormonal, behavioural, and cognitive signals (Strubbe and Woods, 2004). Despite the scarcity of research examining these pathways specifically in BED, a review looked at different studies investigating individuals with a loss of control while eating, also a key feature of BED, found lower levels of fasting ghrelin, elevated leptin levels, and altered postprandial ghrelin responses (Yu et al., 2021). Ghrelin, a hormone secreted by the stomach, has been shown to stimulate appetite by signalling hunger to the hypothalamus. Typically, these levels exhibit an increase prior to meals and a subsequent decrease following the ingestion of food (Cummings et al., 2005). In contrast, leptin, primarily secreted by adipose tissue, signals satiety and energy sufficiency, thereby reducing appetite and promoting energy expenditure (Friedman and Halaas, 1998). Dysregulation of these hormones may contribute to the pathophysiology of BED.

Dysregulation at the neural level could also contribute to the development of BED. Key structures in the brain might include the hypothalamus in its interaction with the nucleus accumbens, corticostriatal circuits (Voon, 2015), and the striatum (Avena et al., 2011). These structures influence the body's motivation and reward systems (Volkow et al., 2017, Kenny, 2011, Morton et al., 2014). The most robust evidence supporting this assertion can be found for the corticostriatal circuit, which exhibits changes that appear to be associated with the severity of BED symptoms (Donnelly et al., 2018). Various studies have also shown that, compared to people with obesity and without BED, individuals with BED also exhibit characteristic neural activation patterns in tasks involving inhibitory control and reward processing (Steward et al., 2019, Hege et al., 2015, Wolz et al., 2017). Imaging techniques also reveal differences between individuals with BED and both overweight and healthy control groups. A functional MRI Stroop color-word interference task showed an increased inhibitory effect on the brain regions in the ventromedial prefrontal cortex, the inferior frontal gyrus, and the insula (Balodis et al., 2013). The Stroop task is used in research to assess deficits in cognitive control and inhibitory abilities

(Kropotov, 2016), which seem to be compromised in BED (Balodis et al., 2013). Participants must name color words whose font color either matches (congruent) or does not match (incongruent) the word content. Increased interference with incongruent stimuli indicates greater cognitive strain and can be considered an indicator of the ability to suppress stimuli (Kropotov, 2016). Individuals with BED show functional and structural changes in frontal and striatal brain regions involved in impulse control, reward processing, and emotion regulation (Frank et al., 2021). Disruptions in these neural networks cause negative affects which could contribute more frequently to loss of control and binge eating (Giel et al., 2022a). Emotion regulation models and interpersonal theories emphasize that unpleasant emotions and social problems in particular could be a core mechanism in the development of binge eating (Donnelly et al., 2018). The neurobiological changes could contribute to the development or maintenance of binge eating, especially when eating behaviour serves as a coping strategy for short-term relief of negative effects (Schaefer et al., 2020).

Two theoretical models have been advanced to explain the relationship between emotional and social factors and binge eating. The emotion regulation model hypothesises that negative emotional states, including stress, sadness and inner tension, can trigger binge eating episodes by prompting individuals to use food as a maladaptive coping strategy (Leehr et al., 2015). The interpersonal model suggests that interpersonal difficulties, encompassing social conflicts or a paucity of social support, serve as key sources of such negative affect, thereby indirectly contributing to disordered eating behaviour (Ansell et al., 2012). These mechanisms often reinforce each other, meaning that emotional eating may offer temporary relief but can exacerbate both emotional distress and social challenges over time.

Disruptions in cognitive functioning have been recognized in individuals with BED. Among the most consistently reported deficits are impairments in decision-making, inhibitory control, and cognitive flexibility (Kessler et al., 2016, Giel et al., 2017, Schag et al., 2013, Iceta et al., 2021). These cognitive

impairments have been observed not only in individuals with elevated BMI, but also in those with normal weight, suggesting that such dysfunctions are not solely attributable to obesity (Grant and Chamberlain, 2020, Oliva et al., 2020). Furthermore, cognitive deficits in BED have been linked to increased disorder severity and a higher incidence of general psychopathology, including anxiety and depressive symptoms (Grant and Chamberlain, 2020). These cognitive difficulties are also associated with worse therapeutic outcomes (Lucas et al., 2021).

In the context of attentional processes, individuals with BED exhibit a bias toward food-related cues when compared to healthy controls (Kessler et al., 2016). Notably, patients with BED tend to make riskier decisions in tasks that involve decision-making under ambiguity, compared to individuals with obesity without BED and those of normal weight (Mestre-Bach et al., 2020). Another documented feature is delay discounting, where individuals with BED more frequently choose immediate rewards over delayed rewards, particularly when influenced by emotional states. This tendency is more pronounced than in healthy individuals or in those with restrictive eating disorders such as AN (Steward et al., 2017). Encouragingly, some of these cognitive impairments appear to be modifiable through intervention (Schag et al., 2021).

However, emerging evidence indicates that comorbid psychopathology, especially depressive symptoms, may play a more influential role in determining treatment outcomes than cognitive dysfunction alone (Dingemans et al., 2020). The current evidence reinforces the multifactorial nature of BED (Giel et al., 2022a). These overlapping factors may contribute to the onset and maintenance of the disorder, as well as shaping its clinical complexity and treatment responsiveness. A deeper understanding of these mechanisms may pave the way for more targeted and effective prevention and intervention strategies, tailored to the heterogeneous presentations of individuals affected by BED.

1.1.3 Comorbidities common in BED

BED is frequently associated with other health conditions as a substantial number of individuals diagnosed with BED also meet criteria for other mental disorders. About 79% of people with BED have at least one cooccurring mental health condition, with nearly 49% having three or more. The most common comorbidities are anxiety disorders (56.1%), mood disorders (46.1%), disruptive behaviour disorders (25.4%), and substance use disorders (23.7%). Within these categories, phobias are the most prevalent anxiety disorder, major depressive disorder is the most common mood disorder, and alcohol use disorder represents the leading form of substance use disorder (Mars et al., 2025, Kessler et al., 2013). Although anxiety disorders, mood disorders, disruptive behaviour disorders, and substance use disorders are common in the general population, their prevalence is substantially higher among individuals with BED. Even when compared to individuals with similar body weights, those diagnosed with BED demonstrate a higher prevalence of mental disorders (Welch et al., 2016). Furthermore, suicidal behaviors must be addressed. A survey conducted in the USA with 361,710 individuals revealed that 22.9% of those with BED had attempted suicide, compared to only 4.85% of those without BED. This signifies a fourfold elevated risk of suicidal behaviors in individuals diagnosed with BED (Udo et al., 2019). On top of that, recent research indicates that eating disorders are an independent risk factor for suicide, regardless of other mental health issues. The paper suggests that clinicians and mental health providers should develop suicide prevention strategies that target eating disorders themselves, demonstrating the seriousness of the issue (Chen et al., 2025).

Moreover, in addition to BED, there are frequently significant somatic comorbidities, including obesity, diabetes mellitus, and cardiovascular disease (Giuliano and Cottone, 2015, Wilfley et al., 2011, Goran et al., 2003). Therefore literature shows that BED significantly impacts both mental and physical health.

1.1.4 Recognizing BED as cause for Obesity

Obesity is a chronic, multifactorial disease characterised by the excessive accumulation of body fat. Its global prevalence has increased dramatically as it has more than doubled since 1990, affecting 16% of adults in 2022 (Islam et al., 2024). The condition is most commonly diagnosed using body mass index (BMI). BMI is defined as a numerical value calculated from an individual's weight in kilograms divided by their height in meters squared ($\text{BMI} = \text{weight (kg)} / \text{height (m}^2\text{)}$). It serves as an indicator of an individual's level of adiposity. A value of 30 kg/m^2 or higher in adults is classified as Obesity. A BMI between 25 and 29.9 kg/m^2 is defined as overweight. While BMI remains the primary diagnostic tool, additional assessments such as waist circumference and waist-to-hip ratio offer further insight into fat distribution and the associated health risks (World Health Organization, 2024).

Recognising its different forms, the ICD-11 classifies obesity as a disease in its own category, rather than just a risk factor, and distinguishes several subtypes: obesity due to energy imbalance (5B81.0); drug-induced obesity (5B81.1); other specified forms (5B81.Y); and unspecified cases (5B81.Z). In instances where an individual exhibits both obesity and an underlying eating disorder, such as BED, ICD-11 does not categorise BED as a subtype of obesity. Instead, two distinct diagnostic codes are applied: 6B82 for BED and 5B81.0 for obesity due to energy imbalance. This is due to the acknowledgement that both a mental health condition and a physical health state may cooccur (World Health Organization, 2019, Claudino et al., 2019). This classification highlights the importance of considering the clinical context of a diagnosis, including any underlying causes.

Obesity usually develops as a result of a sustained energy surplus, where calorie intake exceeds expenditure. However, this process is influenced by a wide range of interacting factors, including genetic predisposition, environmental conditions, psychosocial stressors, psychological factors and certain medications (Masood and Moorthy, 2023). Obesity significantly

increases the risk of various chronic diseases, including type 2 diabetes, cardiovascular disease, musculoskeletal disorders, sleep apnoea and reproductive health issues (Welsh et al., 2024). In many parts of the world, obesity has overtaken underweight as the more prevalent concern, representing a leading cause of preventable illness and death (Schwinger et al., 2020, Chooi et al., 2019, 2016). The impact of obesity on quality of life, its contribution to rising healthcare costs, and the strain it places on public health systems make the prevention and management of obesity an urgent global priority (Dall et al., 2024, Bjornson et al., Janić et al., 2025).

As previously stated, BED has been shown to result in substantial weight gain, thereby functioning as a contributing factor in the development and maintenance of obesity (Blomquist et al., 2011). Although it has considerable negative impacts, the condition is still often overlooked by healthcare professionals and the general public. (McCuen-Wurst et al., 2018).

Despite its status as the most common eating disorder, BED still remains underdiagnosed and inadequately treated (Kornstein et al., 2016). Public perception often minimizes its severity, viewing it as less disabling and easier to manage than other eating disorders. In addition, people with BED often face stigma because the condition associated with a lack of self-discipline by the general public. However, awareness among health care professionals has increased in recent years (Reas, 2017).

From a clinical perspective, identifying the specific aetiology of obesity is critical to determining the most effective treatment approach. Given the heterogeneity within the obese population, it is essential to study potential subgroups, as different underlying causes may require different therapeutic strategies. A nuanced understanding of these subgroups will likely facilitate more targeted and evidence-based interventions (Takeshita et al., 2024).

1.1.5 Treatment Challenges in BED

Several treatment approaches for BED are currently available. International guidelines (National Institute for Health and Care Excellence, 2017, Hay et al., 2014, Hilbert et al., 2017) recommend evidence-based options such as guided self-help, psychological therapies especially cognitive behavior therapy as well as interpersonal psychotherapy and dialectical behavior therapy as well as pharmacological treatments. The latter may involve second-generation antidepressants, central nervous system stimulants produgs like lisdexamfetamine, anticonvulsants such as topiramate and zonisamide or anti-obesity medications such as orlistat (Giel et al., 2022a).

Despite the range of interventions available, a considerable proportion of individuals diagnosed with BED do not demonstrate sustained improvement (Hilbert et al., 2020). Even after initial recovery, the challenge of relapse persists, with a recurrence observed in 26% of patients with diverse eating disorders. The highest incidence of relapse was noted in patients with BN (31%), while the lowest rate was observed in those with binge eating disorder (10%) (Solmi et al., 2024). It is important to note that recovery over time in treated patients differs by diagnosis: while recovery rates in AN, BN are generally improving the longer patients are followed, BED shows the opposite trajectory. For BED, the recovery rate was 57% at less than two years of follow-up, but this dropped to 16% after six to eight years, indicating that maintaining remission is particularly difficult (Solmi et al., 2024). These findings underscore the necessity for early intervention, prior to the establishment of a network of psychological, behavioural and physical symptoms that prolong obesity and enhance it resistant to treatment (Wang et al., 2019, Monteleone et al., 2022, de Vos et al., 2021).

On the other hand, the course of untreated BED demonstrates its chronic nature. At 2.5 years following the initial assessment, 61.3% of patients still met full criteria for BED, 23.4% were classified as subthreshold, and only 15.3% had achieved remission (Javaras et al., 2024). At five years, 45.7% of patients continued to meet full criteria, 32.6% were subthreshold, and 21.7% were in

remission. The findings suggest that symptoms of BED may evolve over time. In addition, full remission often requires a prolonged period of time, and relapses are common. In adults with a higher BMI, BED tends to improve gradually, though without intervention, this condition is unlikely to resolve. (Javaras et al., 2024).

In view of these limitations, it is acknowledged that current treatment options may not be adequate for all patients. It is evident that further work is required to expand the available interventions. A broadening of treatment approaches could have the potential to enhance long-term recovery and reduce the likelihood of relapse.

Even with the substantial burden of BED, research into eating disorders remains severely underfunded in comparison with other psychiatric conditions of similar prevalence and disease burden. Furthermore, publications from this field are underrepresented in high-impact journals (Haynos et al., 2024). A more substantial and precise body of evidence is therefore imperative, not only to refine existing interventions but also to support innovation in treatment development, ensuring that this vulnerable patient population is better served in both research and clinical contexts.

1.2 DNA Methylation as a Mediator Between Genes and Environment

The development of eating disorders reflects a dynamic interaction between genetic susceptibility and environmental exposures. Twin studies have provided substantial insights into this relationship, highlighting the high heritability of these conditions. Heritability coefficients for conditions like AN, BN and BED have been estimated, with values ranging from 32% to 76%, 28% to 83%, and 39% to 45%. Therefore current research suggests that the development of eating disorders is influenced by genetic factors, but also shows that non-shared environmental factors (for example, a stressor affecting only one twin) play a more significant role than shared environmental factors (such as the family environment) (Wade and Bulik, 2018). This interplay between genetic

predisposition and environmental exposure suggests that additional biological mechanisms may mediate these effects.

The field of epigenetics provides a useful way of understanding the interaction between genes and the environment. Epigenetics is the study of changes to genes that occur without alterations to the DNA sequence itself. However, it should be noted that not all epigenetic modifications are stable. Epigenetic changes can be reversible or permanent and therefore hereditary. Research in this field has revealed the dynamic nature of epigenetic marks, showing that they can vary significantly across cell types, age groups, and in response to environmental factors such as lifestyle and smoking (Joehanes et al., 2016, Horvath and Raj, 2018). A number of mechanisms have been identified as potential contributors to epigenetic change, including histone modification, non-coding RNA sequences, and DNA methylation (Dupont et al., 2009).

This thesis employs the epigenetic mechanism of DNA methylation as subject under investigation. DNA methylation involves the addition of a methyl group to the 5' position of a cytosine base. This modification occurs when a cytosine is followed by a guanine nucleotide in the linear DNA sequence. The methyl group is transferred via a DNA methyltransferase enzyme, forming 5-methylcytosine (Razin and Riggs, 1980, O'Donnell and Meaney, 2020, Deaton and Bird, 2011). Cytosine followed by guanine in the 5' to 3' direction, known as CpG sites, occur at a significantly lower frequency in the genome than would be expected by random chance. Given that there are four genetic bases (adenine, guanine, thymine, and cytosine) the expected frequency of CpG dinucleotides is 6.25%. However, the observed frequency of CpG dinucleotides in the genome is considerably lower, with only 0.88% of sites showing a CpG dinucleotide. This discrepancy is primarily attributed to a methylation-induced mutational bias (Deaton and Bird, 2011). It has been estimated that 70-80% of CpG cytosines are methylated and therefore 5-methylcytosine, which is highly susceptible to spontaneous deamination. This process converts 5-methylcytosine to thymine, resulting in a T-G mismatch. In contrast to unmethylated cytosines, which undergo deamination to uracil and are efficiently repaired, T-G mismatches

frequently avoid correction or are mistakenly resolved to T-A during DNA replication. This phenomenon, when repeated over many generations, results in the permanent conversion of CpG to TpG or CpA, leading to a progressive loss and therefore explaining the low percentage of CpG dinucleotides sites in the genome. This phenomenon is not confined to CpG sites and applies to methylated cytosines in general (Razin and Riggs, 1980, Gardiner-Garden and Frommer, 1987). Nevertheless, CpG sites frequently occur in clusters known as CpG islands, which are often located near gene promoters or other regulatory areas and play a crucial role in gene regulation (Blackledge and Klose, 2011, Deaton and Bird, 2011). The role of DNA methylation in general is dependent on its position within the genome. The methylation of DNA in gene regulatory regions where transcription factors bind is typically associated with transcriptional repression. However, the relationship between DNA methylation and transcription in regions such as the gene body is more complex. This relationship can also be positively associated (Wagner, 2025, Maunakea et al., 2010, Shenker and Flanagan, 2012).

Because DNA methylation is both stable enough to produce lasting biological effects and flexible enough to respond to environmental influences, it serves as a key mechanism linking genetic makeup and environmental factors. This role may make DNA methylation particularly relevant to the mechanisms underlying eating disorders, which exhibit distinctive features such as sex differences, adolescent onset, and sensitivity to early-life stressors, making them well-suited for epigenetic investigation (Volpe et al., 2016, Hübel et al., 2019b).

The development of eating disorders reflects a complex interplay between genetic susceptibility and environmental exposures, with epigenetic mechanisms such as DNA methylation offering a plausible pathway for this interaction. DNA methylation provides a lens through which stable yet environmentally responsive modifications can influence gene regulation and, as a consequence, potentially behavior, thereby enhancing our understanding of the biological foundations of these conditions.

1.2.1 Current Knowledge on DNA Methylation in Eating Disorders and Obesity

The development of eating disorders is influenced by a multifaceted interplay of genetic and environmental factors (Yao et al., 2021). Mounting evidence indicates that alterations in DNA methylation patterns across the genome play a substantial role in their pathogenesis (Iranzo-Tatay et al., 2022). However, research in this area remains limited. Many studies have small sample sizes, heterogeneous participants, and focus on candidate genes. Most are cross-sectional, making it difficult to determine whether epigenetic changes precede or follow the disorder. Large scale, longitudinal studies with standardized methods are largely lacking, leaving gaps in understanding epigenetic mechanisms in eating disorders (Käver et al., 2024, Booij and Steiger, 2020).

Among the eating disorders, AN has received the most extensive research attention with regard to epigenetics, with studies encompassing epigenome-wide association studies, global methylation measurements, and candidate gene approaches (Hübel et al., 2019b). In AN, altered DNA methylation has been identified in ten genes (DRD2, GHSR1a, HDAC4, LEP/LEPR, NR1H3, OXTR, SLC6A3/DAT, SLC6A4, SNCA, TNXB) which are involved in key biological pathways, including neurotransmitter regulation, hormonal control of appetite and metabolism, stress response, and chromatin remodeling. The majority of these genes exhibit hypermethylation in patients with AN compared to healthy controls, with the exception of LEP and SLC6A4 (Käver et al., 2024). AN represents the most studied ED in epigenetic research, yet the overall body of evidence remains limited, highlighting a substantial gap in research in general.

Notably, research on BN remains comparatively limited. However, a study has underscored the role of brain derived neurotrophic factor, a neurotrophin implicated in energy balance and food intake regulation, which exhibits reduced serum levels in both AN and BN (Monteleone et al., 2004, Nakazato et al., 2003). The investigation revealed increased BDNF promoter methylation in women diagnosed with BN compared to healthy controls, with particularly elevated levels observed in those with comorbid borderline personality disorder or a history of childhood abuse. These findings additionally underscore the

notion that adverse life experiences may have a further influence on epigenetic changes (Thaler et al., 2014).

Despite the limited attention BED has received in this field, it is the primary focus of this thesis, and therefore, studies on BED will be discussed in greater detail. A comprehensive analysis examined genome-wide DNA methylation differences between BN (n=25) and BED (n=21), involving a total of 46 individuals. Remarkably, this study unearthed 11 differently methylated sites, each bearing genome-wide significance. Crucially, 9 of these sites exhibited hypomethylation in individuals diagnosed with BED. This highlights the existence of epigenetics variations between BED and BN (Rodríguez-López et al., 2021).

Related evidence comes from studies investigating weight and metabolic traits in non BED populations. For example, the Illumina Infinium HumanMethylation450 BeadChip was used to examine DNA methylation differences in 60 lean and 60 obese young women. While this study did not specifically include individuals with BED, it revealed that hypomethylation was more prevalent in obese participants, identifying 10 differentially methylated CpG sites linked to 8 gene loci emphasizing the epigenetic component in obesity-related traits and metabolic risk factors. This finding underscores the influence of gene environment interactions mediated by epigenetic factors in the context of obesity (Kvaløy et al., 2018).

In addition, research focusing directly on BED shows that males diagnosed with BED exhibit significantly lower levels of OXTR DNA methylation compared to males without BED (Giel et al., 2022b). This provides further weight to the argument that epigenetic differences might extend beyond behavioral disparities between individuals with and without BED.

A recent rat study investigated the interaction between stress and food restriction with regard to gene expression and epigenetic changes in specific brain regions (Mercante et al., 2024). In this model, female rats were exposed

to cycles of food restriction in combination with frustration stress. During these cycles, the rats could see but not access highly palatable food for a period of 15 minutes. The control groups experienced only food restriction, only stress, or neither. After the observation of repeated episodes of binge like behaviour, researchers extracted DNA from several brain regions for the purpose of measuring gene expression. Additionally, DNA methylation patterns on gene promoters were examined. The study revealed that rats exhibiting binge-eating behavior demonstrated augmented expression of the adenosine A2A receptor in the amygdala and ventral tegmental area, while the dopamine D2 receptor exhibited a decrease in the nucleus accumbens. These alterations were accompanied by site-specific DNA methylation changes: in the ventral tegmental area for adenosine A2A receptor, and in the amygdala and caudate putamen for dopamine D2 receptor. The results of this study demonstrate the importance of epigenetic mechanisms in the regulation of A2A receptor and dopamine D2 receptor during binge-eating behaviour (Mercante et al., 2024).

While the primary focus of this thesis is on DNA methylation as an epigenetic modification, it is important to acknowledge a study on histone modifications to ensure comprehensive understanding. A study on rodents has indicated a selective reduction in the expression of the fatty acid amide hydrolase gene, which is part of the endocannabinoid system. The observed downregulation was accompanied by a decrease in histone H3 acetylation at lysine 4 on the gene's promoter. Notably, no substantial alterations were detected in other components of the endocannabinoid system across the brain regions examined, nor in additional epigenetic modifications at the faah promoter like DNA methylation or histone H3 lysine 27 methylation. This suggests that the effects seen are likely to be a selective modulation (Pucci et al., 2019).

In summary, the evidence from these studies suggests that epigenetic patterns may differ between BED and obesity, leading to Hypothesis 1 proposing that there could be a DNA methylation difference at a specific gene between the two conditions.

1.2.2 Observations of Sex -specific Differences in DNA Methylation Patterns

The prevalence of BED exhibits notable sex differences. According to the available data, the global prevalence of BED for the 2018–2020 period is estimated to range from 0.6% to 1.8% among adult women and from 0.3% to 0.7% among adult men (Giel et al., 2022a). This pronounced difference in the prevalence of BED between the sexes may reflect underlying epigenetic variation. Differential methylation at specific genes could explain the disparities observed in the likelihood of men and women developing BED.

On an epigenome wide level, recent research has investigated into the broader landscape of sex-specific DNA methylation patterns. A study involving a large European cohort of 1799 individuals identified and validated 1184 CpG sites with different DNA methylation between men and women. These sex-related differences in DNA methylation were distributed across all autosomes and were concentrated in CpG island shores, suggesting substantial impact and importance in future studies' design and analysis (Singmann et al., 2015).

More on, sex-specific DNA methylation changes have also been observed in the context of aging. This indicates that many CpG sites with sex-specific DNA methylation patterns also change with age, underlining the intricate interplay of these factors (Yusipov et al., 2020). Studies directly comparing DNA methylation patterns in males and females have found slightly higher methylation levels in males for most CpGs (El-Maarri et al., 2007). On the other hand, a study of over 700 children measured at ages 9 and 15 found that most DNA methylation sites showed higher methylation in females. Particularly, this study also showed that these sex differences in methylation were stable over time (Reiner et al., 2023).

These contradictory findings suggest that sex-specific DNA methylation patterns are complex and currently lack a consistent or clear pattern across studies.

Furthermore, even among individuals with identical genetic backgrounds (therefore monozygotic twins), sex-related differences in whole-genome DNA methylation of CpG islands have been identified. It was demonstrated that differences in DNA methylation levels within pairs of twins differ between males and females, with males showing greater variability in autosomal CpG islands and females showing greater variability in CpG islands on the X chromosome (Watanabe et al., 2016). This suggests potential associations with sex-specific susceptibility to certain diseases, which could have implications for understanding the sex disparity observed in eating disorders.

The effects of sex on DNA methylation have been demonstrated in the context of another mental illness like schizophrenia, Post traumatic stress disorder and Depression (Uddin et al., 2013). In an epigenome-wide association study, researchers analyzed and compared peripheral blood DNA methylation data from 1,519 schizophrenia cases and 1,723 controls. The analysis was conducted in a sex-specific, sex-stratified, and sex-adjusted manner. The study's findings indicate the presence of an interaction between sex and schizophrenia status on DNA methylation. It is also hypothesized that this could serve as an effective approach for the implementation of precision psychiatry and the development of stratified treatments for schizophrenia (Tesfaye et al., 2024).

The research conducted on individuals with BED, particularly in the context of epigenetics and the specific focus on sex differences is minimal. For the OXTR gene, no differences in DNA methylation were observed in females with BED, and no overall sex differences were found, whereas males with BED exhibited lower OXTR methylation than males without BED. (Giel et al., 2022b). This highlights that epigenetic differences due to sex may contribute to the manifestation of BED, and further research is needed to elucidate the mechanisms underlying these sex-specific patterns.

In conclusion, sex differences have been demonstrated in BED. These differences may suggest underlying epigenetic variations that contribute to this

sex-based contrast. Recent research has also stressed the significance of sex-specific DNA methylation patterns across various mental health conditions. This leads to Hypothesis 2, proposing that there may be a DNA methylation difference at a particular gene between males and females with BED.

1.2.3 Potential Influence of Eating Behaviour or Body-Mass-Index Scores on DNA Methylation

The impact of dietary factors on DNA methylation is an important area of research (García-García et al., 2024, Kadayifci et al., 2018). Nutrient intake, particularly substances like folate, B12, and choline, plays a crucial role in shaping one-carbon metabolism, a process vital for DNA methylation. Emerging evidence in humans suggests that specific nutrients in a diet have the potential to influence not only DNA methylation but also brain function (McGarel et al., 2015, Amenyah et al., 2020b, Steiger and Booij, 2020). Also a relationship has been demonstrated between various nutritional factors and dietary patterns and DNA methylation in genes involved in glucose homeostasis, lipid metabolism, and obesity-related genes across various life stages and generations (Meir et al., 2023). This suggests that nutritional factors may directly affect DNA methylation levels in individuals with eating disorders.

Furthermore, the results of epigenome-wide association studies that have identified 187 genetic loci where DNA methylation is significantly associated with BMI (Wahl et al., 2017). BMI is widely used as a risk factor for assessing the development and prevalence of numerous health conditions (Nuttall, 2015, Zierle-Ghosh and Jan, 2025). The authors state changes in DNA methylation are primarily a consequence of BMI rather than the cause (Wahl et al., 2017). In contrast, the findings of another study have indicated a correlation between specific DNA methylation sites in umbilical cord blood and the change in children's BMI over time, or what is otherwise referred to as longitudinal trajectories. Another study analysed the DNA methylation of around 480,000 sites in human adipose tissue and then compared these methylation patterns with those measured in matched blood samples from 96 men and 94 women. The study identified 2,825 genes for which DNA methylation correlated with BMI

(Rönn et al., 2015). Furthermore, it was also shown that the relationship between DNA methylation and BMI varies by sex, indicating the presence of sex-specific epigenetic regulation (Yaskolka Meir et al., 2023).

A review of the extant literature on individuals diagnosed with BED reveals an absence of a clear correlation between BMI and DNA methylation levels. For instance, a study examining the association between DNA methylation and BMI found that no CpG sites reached genome-wide significance, even though the majority of sites were hypomethylated in the BED group tested (Rodríguez-López et al., 2021). Furthermore, mean DNA methylation levels did not demonstrate a significant relationship with BMI or self-reported eating disorder pathology. The findings indicate that, on average, DNA methylation levels may not exhibit a strong correlation with BMI (Giel et al., 2022b).

In summary, research has shown that nutritional factors and therefore eating behaviour can influence DNA methylation, although the relationship between DNA methylation and BMI appears to be complex and not uniformly observed across all CpG sites or individuals. Nevertheless, the intricate interplay between dietary factors and DNA methylation necessitates continued investigation to fully comprehend these relationships. This forms the basis for Hypothesis 3, proposing a correlation between DNA methylation levels at a specific gene and BMI.

1.3 The Nociceptin opioid peptide receptor (NOP)

Nociceptin opioid peptide receptor (NOP) is a member of the opioid receptor family. Initially characterized as Opioid Receptor Like-1, NOP is a G protein-coupled receptor that binds to its corresponding 17-amino acid peptide, Nociceptin/Orphanin FQ (N/OFQ), thereby initiating a signal transaction from outside the cell to the inside (Meunier et al., 1995, Reinscheid et al., 1995). In a functional level NOP activation also influences potassium and calcium channels, thereby reducing neuronal activity (Connor et al., 1996a, Connor et al., 1996b, Ikeda et al., 2000).

As a member of the opioid receptor family, NOP shares significant structural and genetic similarities with the classical mu, delta, and kappa opioid receptors, with an overall sequence homology of ~60% (Thompson et al., 2012). All are Class A (rhodopsin-like) G protein-coupled receptors with conserved motifs in their transmembrane and intracellular regions, respond to peptide ligands, and signal through G-protein-mediated pathways (Toll et al., 2016). Classical opioid receptors bind endogenous opioids such as endorphins, enkephalins, and dynorphins, but for the most part, they do not bind N/OFQ (Che and Roth, 2023). Likewise, NOP selectively binds N/OFQ and, for the most part, does not bind or respond to morphine-like compounds or typical opioid antagonists such as naloxone (Thompson et al., 2012). While very high concentrations of naloxone (>10 μ M in vitro) can weakly interact with NOP, normal therapeutic doses have no effect, reflecting low-affinity binding (Spagnolo et al., 2008). These pharmacological differences arise from structural variations in the binding pockets of NOP (Thompson et al., 2012).

NOP regulates a broad range of distinctive physiological processes, including pain, reward and mood, which overlap with the functions of classical opioid receptors (Al-Hasani and Bruchas, 2011, Ubaldi et al., 2021, Zhang et al., 2020b). However, classical mu, delta, and kappa receptors are also additionally involved in the regulation of the gastrointestinal system, respiratory depression, endocrine modulation, and immunomodulation (Herman et al., 2025). One physiological role that is thought to be uniquely regulated by the NOP receptor rather than the classical opioid receptors is the homeostatic control of food intake and body weight (Farhang et al., 2010). While classical opioid receptors, particularly the mu opioid receptor, are thought influence hedonic and reward-driven eating (Nummenmaa et al., 2018), NOP is hypothesised specifically involved in metabolic state dependent feeding and recovery from negative energy balance (Przydzial et al., 2010).

As the presence of NOP has been found in several brain regions, it has been recognised as a modulatory system that is also functionally connected to various pathological processes. Research has indicated an association between

alterations in NOP signalling are associated with alcohol abuse (Huang et al., 2008), depression (Post et al., 2016), post-traumatic stress disorder (Narendran et al., 2019), opioid dependence (Briant et al., 2010), and chronic pain (Toll et al., 2019).

Overall, NOP is a standout member of the opioid receptor family. Although it shares structural and mechanistic features with classical receptors, it exhibits distinctive pharmacological and functional properties. Dysregulation of NOP signalling has been associated with various mental health and pain related conditions, suggesting its potential as a target for future research.

1.3.1 Potential Role of NOP in BED

The role of NOP in regulating food intake, particularly focusing on its relevance to binge eating behaviour is explored. Key findings from both animal studies and clinical trials are highlighted.

Inhibition of NOP has been demonstrated to effectively hinder overeating in both rats and mice, signifying its function in regulating food intake. Conversely, administering a NOP receptor agonist directly into the brains of rats led to augmented food consumption, further emphasizing the importance of this receptor in short term eating behaviour (Economidou et al., 2006, Hardaway et al., 2016).

Initial investigations into NOP function began with single shot studies using N/OFQ, which acts as a ligand for NOP. This significantly increases food intake in rodent. Even more they were able to show that higher doses lead to more food intake. Administration of naloxone, an opiate antagonist, effectively revoked this effect, confirming the involvement of the opioid system in this modulation of feeding behaviour (Pomonis et al., 1996).

Further studies examined the effects of N/OFQ on food intake, as well as its temporal dynamics, in rodents. These studies revealed that N/OFQ significantly

increased food intake without substantially affecting water intake. The effects of N/OFQ lasted up to 45 minutes, demonstrating that it temporarily but effectively influences eating behaviour (Micioni Di Bonaventura et al., 2019, Polidori et al., 2000).

In view of the evidence obtained, researchers subsequently expanded the investigation to incorporate longer term administration as well as more controlled conditions. In a study administering a 12 day regimen of N/OFQ injections led to heightened food intake and consequent weight gain in rodents. These findings strongly indicate a correlation between NOP activation and alterations in energy balance. This pair-fed study, in which animals received the same amount of food as the vehicle group showed several adverse effects including increased adipose tissue, increased lipogenic gene expression, hyperinsulinemia, hypercholesterolemia, suppressed brown adipose tissue function and decreased body temperature. These findings showcase a multifaceted influence of NOP on energy homeostasis (Matsushita et al., 2009).

Several studies have examined how N/OFQ influences feeding under specific conditions, such as stress or exposure to palatable foods. Research indicates that activation of NOP can modify or even override behavioral responses that were established through conditioning. N/OFQ completely abolished the hypophagic effects induced by electroshocks or intravenous corticotropin-releasing factor injection in rodents. Stress induced feeding, resulting in selective sucrose intake, can be impeded by antagonists in rodents (Ciccocioppo et al., 2001, Ciccocioppo et al., 2002, Ciccocioppo et al., 2003). This suggests a correlation between sugar intake and increased eating (Bulik et al., 2003). Hyperphagic effects of N/OFQ are seen pre- and post-synaptic and have been consistently demonstrated (Farhang et al., 2010).

Research on knockout mice (NOP(-/-)) has been especially insightful. When presented with a choice under conditions of food restriction, NOP KO mice demonstrated a decreased preference for a high-sucrose diet and consumed less of a high-fat diet when no-choice conditions were imposed. However, these responses were not observed when food was freely available (ad libitum

feeding). Furthermore, conditioned place preference to a high-fat diet remained unaffected in NOP KO mice, even under food-deprived conditions, suggesting that there was no alteration in reward responses associated with the consumption of a high-fat diet. After refeeding following food deprivation, NOP KO mice displayed normal increases in plasma glucose and insulin levels. However, multidimensional scaling analysis unveiled disrupted relationships between plasma glucose, insulin, body weight, and plasma leptin, particularly noticeable in fasted mice. The correlation between body weight and plasma leptin was weaker in NOP KO mice. This indicates that endogenous nociceptin may regulate dietary preference independently of motivational, rewarding (Koizumi et al., 2009).

Moreover, NOP(-/-) mice exhibited reduced food intake, particularly during the first 2 hours of free access to food, even when intracerebroventricular N/OFQ was administered, suggesting a role in the regulation of feeding behaviour (Farhang et al., 2010). In addition, studies have replicated the findings of reduced fasting-induced feeding in the presence of N/OFQ (Witkin et al., 2014, Statnick et al., 2016).

Furthermore, studies have examined the neuronal mechanisms underlying NOP, exploring its role in regulating feeding behaviour and metabolism in response to nutritional changes. A high expression of NOP has been identified within the ventromedial hypothalamus (Gehlert et al., 2006), a nucleus well known for its function as a satiety center that inhibits food consumption when activated. Early studies using non-selective physical lesions or pharmacological damage to the ventromedial hypothalamus led to overeating, highlighting its essential role in inhibiting food intake. The ventromedial hypothalamus plays a central role in energy homeostasis, integrating hormonal and neural signals to regulate feeding and maintain body weight. Its diverse neuronal populations contribute to multiple physiological processes, including autonomic output that alters cardiovascular tone and neuroendocrine responses as well as gastric and reproductive functions, highlighting the complexity of its regulatory influence (Chan and Sherwin, 2013, Zhang et al., 2020a). The localization of NOP within

the ventromedial hypothalamus might underscore its importance in mediating N/OFQ's effects on feeding and metabolism, illustrating how specific neural circuits contribute to the control of energy balance and the intricate pathways that underlie these essential behaviours (Chee et al., 2011, Olszewski et al., 2010, Zhang et al., 2020a).

Additionally, researchers have investigated the impact of food deprivation on NOP on gene expression level. The study revealed that food deprivation leads to a reduction in NOP mRNA levels across several brain regions in rats. This indicates that NOP could be dynamically regulated in response to changes in nutritional status, showing its possible importance in the context of feeding behaviour (Rodi et al., 2002).

Moreover, a link has been proposed between food restriction and NOP. Research has demonstrated that food restriction leads to a decrease in NOP mRNA levels in the hypothalamus of rodents. Based on these findings, it has been hypothesized that a potential hypofunction of the NOP system could contribute to the maintenance of BED however, this theory has not yet been confirmed by experimental evidence (Pucci et al., 2016).

The previous section presented an overview of animal studies done on this topic. This section will now shift the focus towards research conducted in a clinical setting on humans.

In a study of a cohort of 16 obese and 25 normal weight women, 20 of whom met the criteria for BN, participants received intravenous infusions of naloxone (an opiate antagonist), bupropion (an opiate agonist), or a saline placebo. BN is an eating disorder characterised by recurrent episodes of binge eating, but compared to BED this is followed by purging behaviours such as vomiting or excessive exercise. In this experimental design, during the drug infusion, the women participated in taste tests that included tasting sweetened dairy products and snacks of varying sugar and fat content. Results showed that naloxone, the opiate antagonist, suppressed hedonic responses to food in all subject groups. Notably, naloxone also reduced the consumption of sweet and high-fat foods,

particularly in individuals with binge eating disorder. However, it did not significantly affect food intake in obese women. In contrast, the opiate agonist butorphanol had no significant effect on hedonic responses or food consumption in any of the participant groups. This suggests that opiate blockade, particularly with naloxone, may hold promise in the clinical management of BED by reducing the consumption of foods associated with binge eating (Drewnowski et al., 1995).

A placebo controlled, double-blind pilot study was conducted to evaluate the effects of naltrexone combined with bupropion (NB) on BED with comorbid obesity. In this study, 22 adult patients with BED were randomized to receive either NB or placebo for 12 weeks. Primary outcomes focused on changes in binge eating frequency and weight percentage from baseline, with secondary outcomes assessing eating disorder psychopathology and depression. Results showed that both the NB and placebo groups experienced significant reductions in binge eating, eating disorder psychopathology, depression, and weight during the treatment phase. Notably, a significantly higher percentage of patients in the NB group (45.5%) achieved a 3% weight loss compared to the placebo group (0%). These findings highlight the potential efficacy of NB in the treatment of BED, particularly with regard to weight loss and improvements in binge eating. In addition, the study suggests that NB is well tolerated in patients with comorbid obesity and BED. However, the need for larger studies with sufficient statistical power is emphasized to determine the full extent of the efficacy of NB in the treatment of BED (Grilo et al., 2021).

The understanding of BED treatment has been advanced by evaluating the efficacy of NB and behavioural weight loss therapy (BWL), both individually and in combination, in individuals with BED comorbid with obesity. The randomized, double-blind, placebo controlled trial included 136 patients who were divided into four groups: placebo, NB, BWL + placebo, and BWL + NB, each of which received 16 weeks of treatment. The study results showed that the BWL + NB group achieved the highest binge-eating remission rate (57.1%), followed by the BWL + placebo group (37.1%), the NB group (31.3%), and the placebo group

(17.7%). NB combination outperformed placebo group in achieving binge eating remission. In addition, participants in the BWL + NB group had the highest percentage of individuals achieving a 5% weight loss (38.2%), followed by the BWL + placebo group (31.4%), the NB group (18.8%), and the placebo group (11.8%). Taken together, these results indicate that both BWL and naltrexone-bupropion are effective in the treatment of binge eating disorder (Grilo et al., 2022).

Despite the fact that a number of individual studies incorporated within this thesis have previously been discussed, a meta-analysis of eight randomised controlled trials furnishes a more robust synthesis of evidence. This Metaanalyses is increases statistical power and providing a more reliable estimate of treatment effects (Cohn and Becker, 2003). The findings suggest that opioid antagonists, like naltrexone, exhibit a substantial reduction in binge-eating frequency and body weight percentage, with moderate to large effect sizes. However, these interventions do not demonstrate a significant impact on binge-eating severity or depressive symptoms. The efficacy of treatment also appears to be moderated by dosage (Fang et al., 2025).

In summary, a compelling line of evidence comes from studies involving NOP, revealing its critical role in the regulation of eating behaviour. Inhibition of the NOP has been shown to effectively suppress overeating in rodents, providing insight into potential therapeutic strategies for BED. On top, injection of NOP agonists into the brain of rats has been associated with increased food intake, further implicating NOP in short term eating behaviour.

In addition, preliminary data of interventions targeting NOP in humans, such as the administration of naloxone, in reducing sweet and high fat food intake in individuals with binge eating disorder, underscores the clinical relevance of this pathway.

Taken together, these findings suggest that regulation of the NOP gene may differ between individuals with BED and those with obesity without BED. Consequently, the NOP gene has been selected as the specific focus for

investigation across all three hypotheses in this thesis, providing a clear framework to examine potential DNA methylation differences related to BED, sex differences, and correlations with BMI.

1.4 Research Objectives

The hypotheses for this thesis were derived from literature on DNA methylation in eating disorders and obesity, include

ing sex-specific differences and BMI associations (Chapters 1.2.1–1.2.3). The NOP gene was chosen to test these hypotheses in a gene-specific context (Chapters 1.3.1).

Hypotheses 1: *DNA methylation levels of the NOP receptor gene differ between individuals diagnosed with BED and BMI-matched control participants without BED.*

Hypotheses 2: *Sex-specific differences exist in DNA methylation levels of the NOP receptor gene among individuals with BED and BMI-matched controls.*

Hypotheses 3: *BMI Scores correlate with DNA methylation levels of the NOP receptor gene.*

2 Material and Methods

In the section "Material and Methods," the selected CpG site are stated, also cohort under examination is introduced. Moreover, the questionnaire used, the workflow incorporating laboratory procedures and the statistical analysis employed to assess the hypotheses are presented in detail. The study was conducted in accordance with the approved ethical principles, as outlined in the project number 966/2021BO2. The positive ethics statement from the Ethics Committee of the University of Tübingen was granted on 17 March 2022.

2.1 Cohort Assessment and Sample Collection

The cohort consisted of individuals diagnosed with BED (BED+) and a comparison group of individuals with obesity but without BED symptoms (BED–). In total, 229 participants were included, with 130 assigned to the BED+ group and 99 to the BED– group.

Participants were recruited between 2014 and 2020 from the inpatient, day-patient, and outpatient services of the Department of Psychosomatic Medicine and Psychotherapy at the University Hospital Tübingen. Eligibility criteria included a diagnosis of obesity or BED and the provision of written informed consent. No specific exclusion criteria were applied. All participants underwent a structured and standardized diagnostic assessment. This included measurement of height and weight by study staff to calculate BMI, as well as a structured clinical examination to confirm or exclude an eating disorder diagnosis according to DSM-IV criteria (American Psychiatric Association, 1994). Self-reported eating disorder pathology was additionally assessed using the Eating Disorder Examination Questionnaire (EDE-Q) (Hilbert and Tuschen-Caffier, 2016). Venous blood samples were collected in EDTA tubes and stored at –80 °C until analysis. The study was approved by the Ethics Committee of the Medical Faculty of the Eberhard Karls University Tübingen, Germany.

The present cohort has previously been the subject of an examination; consequently, the recruitment and assessment methods employed in this study are consistent with those used in the cited study (Giel et al., 2022b).

2.2 Eating Disorder Examination-Questionnaire (EDE-Q)

The Eating Disorder Examination Questionnaire (EDE-Q) is a self-report instrument designed to capture the frequency and severity of eating disorder related behaviours and attitudes in adults and adolescents. It yields scores on four subscales (Restraint, Eating Concern, Shape Concern, and Weight Concern) as well as a total score reflecting overall eating disorder psychopathology. Higher scores indicate greater severity of symptoms (Fairburn et al., 1993). While the EDE-Q can provide a bases for diagnostic evaluation, it is more commonly used to assess the severity of eating disorder symptoms. It is commonly applied in both clinical and research contexts (Berg et al., 2012). For the present study, the German version of the questionnaire was used (Hilbert and Tuschen-Caffier, 2016).

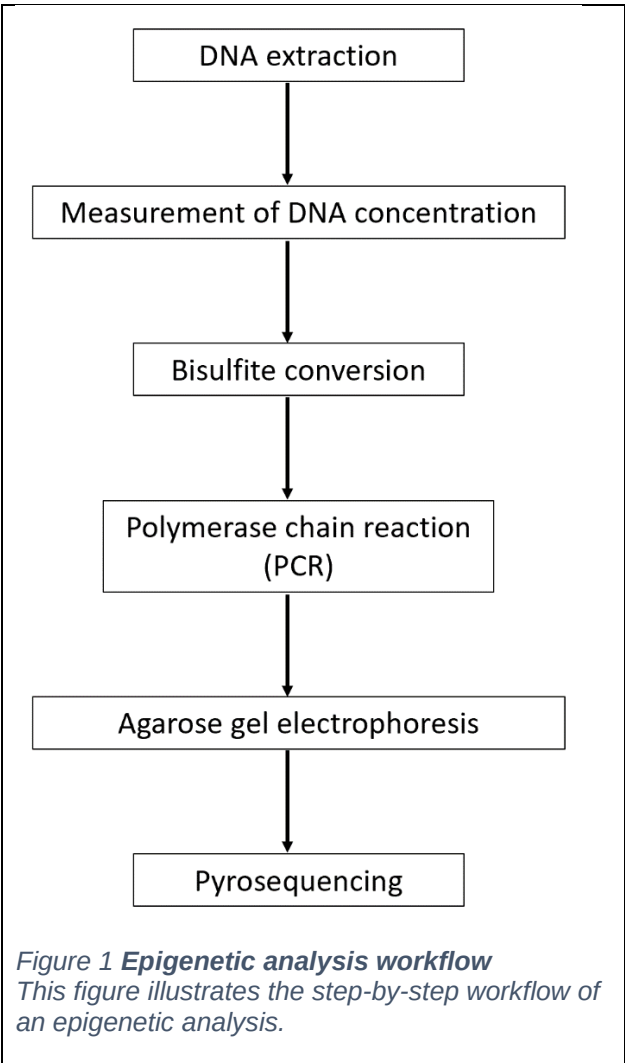
2.3 Selection of the Studied CpG sites

This study aimed to examine the DNA methylation patterns within the NOP gene, located on chromosome 20:62718473-62719016 (hg19). The selection of the studied CpG sites was based on two papers (Ruggeri et al., 2015, Ruggeri et al., 2018). In their research, the group demonstrated that adults with alcohol use disorder exhibited hypermethylation of the NOP through genome-wide methylation analysis. Additionally, the CpG sites were studied in adolescents with regard to binge drinking and early psychosocial stress exposure. Reduced DNA methylation was found in the first intron of the NOP gene.

Substance abuse, particularly alcohol use disorder, is a common comorbidity in BED (Mars et al., 2025). Alcohol use disorder and BED are both characterized by overconsumption. A common thread between the two illnesses is the dysregulation of the reward system, impulsivity, and an overlap with negative emotions such as depression, anxiety, and the experience of guilt (Jeong et al., 2025, Ralph-Nearman et al., 2020, Schulte et al., 2016).

A British study demonstrated that risky drinking and binge eating in young adults frequently occur and often co-occur in the same individuals. Among the 155 students surveyed, 30 exhibited symptoms of both risky drinking and binge eating, highlighting the overlap between these behaviours. 9 reported symptoms of binge eating alone and 73 showed symptoms of risky drinking alone. This pattern suggests that these conditions are not only common individually but also tend to manifest together in a 19% proportion of students (Ralph-Nearman et al., 2020). Based on these observations, we selected these specific CpG sites for further investigation.

2.4 Workflow of the epigenetic analyses



2.4.1 Procedure

Three CpG sites within the first intron of the NOP gene (chromosome 20:62718473-62719016, hg19) were analysed (Figure 2).

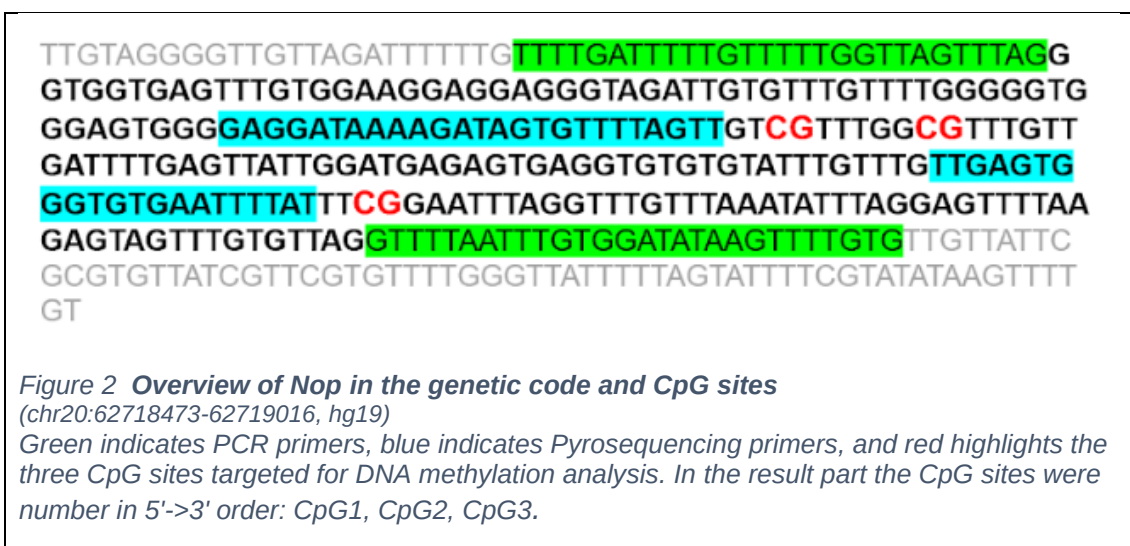
The epigenetic analyses began with the extraction of genomic DNA from EDTA anticoagulated venous blood using the QIAamp DNA Blood Maxi-Kit.

Bisulfite conversion was a key step in the analysis, converting unmethylated cytosine bases into uracil while leaving methylated cytosines unchanged. This treatment allows for the specific examination of DNA methylation at CpG sites.

After bisulfite conversion, PCR amplification was performed to generate sufficient material for downstream epigenetic studies. To validate the success of bisulfite conversion and PCR amplification, agarose gel electrophoresis was applied as a quality check to ensure sample integrity and absence of contamination.

To quantify DNA methylation levels at the specific three CpG sites, pyrosequencing was used. The PyroMark Q24 system, in conjunction with PyroMark GoldReagents, enabled precise quantification of DNA methylation levels in each sample.

Balanced design approaches and control DNA samples with known methylation levels were included in the analysis to ensure the accuracy of the pyrosequencing results. An overview of the workflow is shown in Figure 1.



2.4.2 DNA Extraction and DNA Concentration Measurement

Genomic DNA was extracted from venous blood preserved using EDTA anticoagulant for epigenetic analysis. The QIAamp DNA Blood Maxi-Kit (Qiagen, Hilden, Germany) was used following the manufacturer's recommended protocol.

DNA concentrations were determined using Qubit® dsDNA BR Assay Kits, according to the recommended manufacturer's protocol. DNA standards and samples were quantified for concentration using a Qubit® 2.0 Fluorometer, which enabled accurate quantification of DNA concentrations for subsequent analyses.

2.4.3 Bisulfite conversion

The DNA sample was diluted to a concentration of 50 ng/μL. Next, the extracted DNA was converted by bisulfite treatment using the EpiTect® Fast DNA Bisulfite Kit from QIAGEN, following the recommended protocol recommended by the manufacturer.

To allow sodium bisulfite to react with cytosines, the DNA is denatured to single strands. Sodium bisulfite reacts with unmethylated cytosines through sulfonation, deamination, and desulfonation steps, converting them into uracils. Methylated cytosines (5-methylcytosine) are protected from this conversion and remain as cytosines.

During subsequent PCR amplification, uracils are read as thymines, so originally unmethylated cytosines appear as thymines in the sequence, whereas methylated cytosines remain as cytosines (Figure 3).

This modification allows selective analysis of CpG DNA methylation status.

tttttaatagcacaatgtg^{cg}g^{cg}ctggtgactgg^{ctt}ccctgggggatggctg^{ct}ctgtgagggg^{ctg}ccaga^{cc}ctg^{ct}g^{ct}ttg
 attc^{ct}gtt^{ct}ttg^{cc}ag^{cc}cagggtggtgag^{ct}gtggaaggaggaggtaga^{ct}gtg^{ct}g^{ct}ccctgggggtgggagtgggga
 gga^{ca}aaaga^{ca}gtgtt^{cc}ag^{ct}g^{cg}ctg^{cg}ctggttctgatt^{ct}gag^{ct}g^{ca}tg^{ct}g^{ct}gagatgaggtgtgtattt
 g^{ct}g^{ct}gagtggtgtgaat^{ct}ca^{cccc}ggaa^{ccc}agg^{ct}g^{cc}caata^{ccc}aggag^{ct}ccaagag^{ca}g^{ct}gtg^{ct}aggt^{ct}
 ttaat^{ct}gtgga^{ca}caag^{ct}ctgtg^{ct}gtcatt^{cg}gtgtcat^{ct}gtg^{ct}ttggg^{ct}at^{cc}ctagat^{ct}ctg^{ca}ca^{ca}ag^{ct}ctg
 tg^{ct}gtcatt^{cg}gtgtcat^{ct}gtg^{ct}ttggg^{ct}at^{cc}ctagat^{ct}ctg^{ca}ca^{ca}ag^{ct}ctgtg^{ct}gtcatt^{cg}gtgtcat^{ct}gt
 cgtg^{ct}ttggg^{ct}at^{cc}ctagat^{ct}ctg^{ca}ca^{ca}ag^{ct}ctgtg^{ct}gtcatt^{cg}gtgtcat^{ct}gtg^{ct}ttggg^{ct}at^{cc}ctag



TTTTTAAATAGTATAAATGTGCGGTTTGGTGATTGGTTTTTTGGGGGATGGTTG
 TTTTGTGAGGGGTTGTTAGATTTTTTGTGTTGATTTTTGTTTTGGTTAGTTTAG
 GGTGGTGAGTTTGTGGAAGGAGGAGGGTAGATTGTGTTTGGGGGTGG
 GAGTGGGGAGGATAAAAGATAGTTTTTAGTTGT^{CG}TTTGG^{CG}TTTGTGATT
 TGAGTTATTGGATGAGAGTGAGGTGTGTGATTGTTTGTGAGTGGGTGTGA
 ATTTTATTT^{CG}GAATTTAGGTTTGTGTTAAATATTTAGGAGTTTAAAGAGTAGTTTG
 TGTTAGGTTTAAATTTGTGGATATAAGTTTGTGTTGTTATTCGCGTGTTATCGTT
 CGTGTGTTTGGGTTATTTTAGTATTTTCGTATATAAGTTTGTGTTGTTATTCGCG
 TGTTATCGTTCGTGTTTGGGTTATTTTAGTATTTTCGTATATAAGT

Figure 3 Schematic representation of the NOP gene sequence before and after bisulfite conversion

Cytosine bases are marked in red to set apart the unaltered methylated cytosines from the unmethylated cytosines that are converted into uracil. During PCR, uracils are read as thymines, so unmethylated cytosines appear as thymines.

2.4.4 Polymerase Chain Reaction (PCR)

The PyroMark PCR Kit by QIAGEN was used for the amplification of the target DNA region in this study. The primers were used at a concentration of 10 μ M.

The forward primer

(TTTTGATTTTTGTTTTGGTTAGTTTAG) and reverse primer

(CACAAACTTATATCCACAAATTAAAC) were designed to be (5'->3')

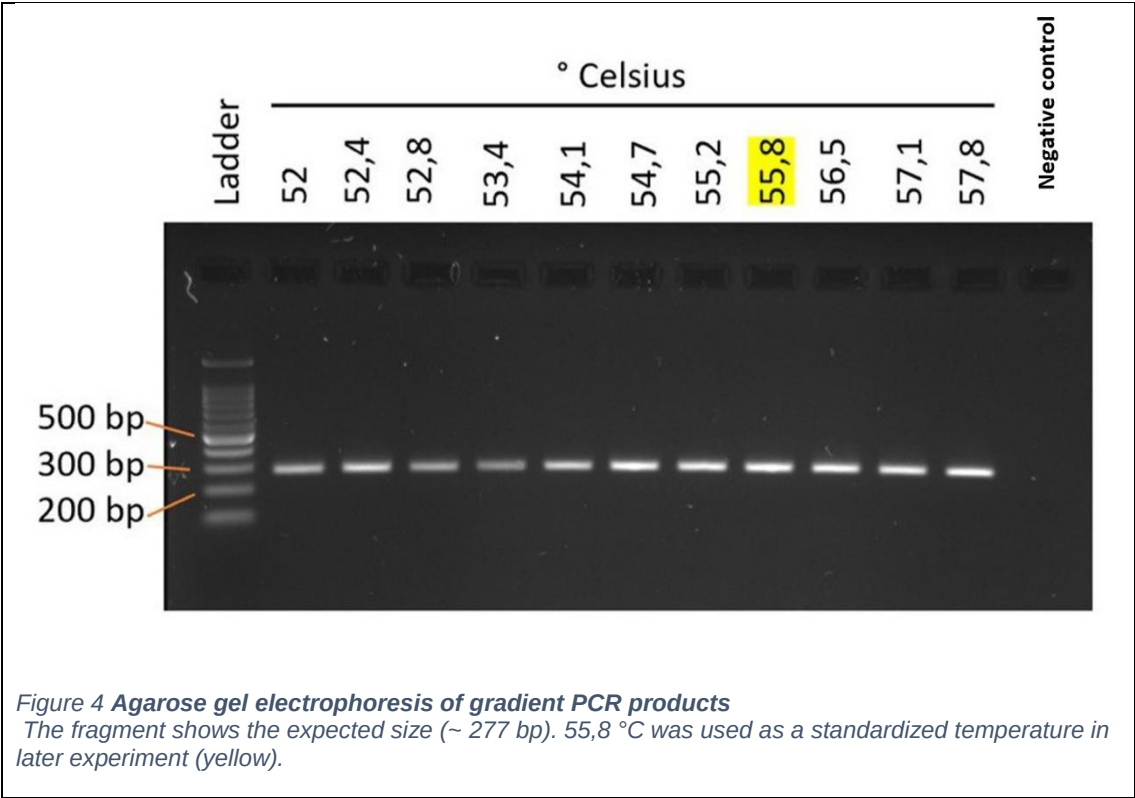
forward (Table 2). The PCR reaction used 1 μ l of the bisulfite-converted DNA as a template, which had undergone bisulfite conversion using 500ng of DNA and 20 μ l of eluate.

Gradient PCR was performed to determine the effectiveness of the primer across the temperature spectrum. The results were consistent, with slightly weaker outputs in the lower temperature ranges. Additionally, this controlled whether the PCR output was the correct size (around 277 bp). A negative control was included (Figure 4).

The PCR program started with an initial denaturation of the DNA strands at 95°C for 15 minutes to ensure efficient strand separation. Subsequently, the cycling phase consisted of 45 cycles of denaturation at 94°C for 30 seconds, which was followed by annealing at 55.8°C for 30 seconds. This allowed the primers to anneal to their complementary DNA strands. The extension phase lasted for 30 seconds at 72°C, leading to the synthesis of new DNA strands from the primers and resulting in the amplification of the target regions. To ensure full extension of all amplicons and reduce non-specific amplification, a final extension step was included at 72°C for 10 minutes. The PCR reaction was then stored at 8°C indefinitely until further analysis.

Table 2 *Primer Sequences (5'->3') for PCR targeting CpG Sites in the experiment*

	Primer Seq (5'->3')
Forward for PCR	TTTTGATTTTGTGTTTTGGTTAGTTTAG
Reverse for PCR	CACAAAACCTTATATCCACAAATTAAAAC



2.4.5 Agarose gel electrophoresis

Agarose gel electrophoresis was used to visualise the PCR products and assess the success of the amplification process. A 2% agarose gel supplemented with 1 μ L of SYBR™ Safe DNA Gel Stain was prepared to improve the clarity of the DNA bands. To determine the size of the PCR products, a 100 bp DNA ladder was added to the gel as a molecular weight marker. 5 μ L of the amplified samples, 5 μ L of the negative control and 5 μ L of the DNA ladder were loaded onto each gel.

Gel electrophoresis, carrying a charge of 100 V and 200 mA, was conducted for 35 minutes, inducing DNA migration that was size-dependent through the gel matrix. Following electrophoresis, the gel was exposed to UV light using an E-BOX VX5 from PEQLAB to visualise the DNA bands. Agarose gel electrophoresis functioned as quality control, validating the specificity and integrity of the PCR products, along with a successful bisulfite conversion, and assuring precise downstream analyses of DNA methylation patterns at specific CpG sites.

2.4.6 Pyrosequencing

DNA methylation levels at specific CpG sites were quantified in this study using Pyrosequencing. The PyroMark Q24 system and PyroMark GoldReagents by Qiagen were utilized in the process. To quantify the level of DNA methylation in each sample, the PyroMark Q24 software version 2.0.6 by Qiagen was used. Three CpG sites were included in the Pyrosequencing assay. Sequencing primers for CpG1, CpG2 (GAGGATAAAAGATAGTGTTTTAGTT) and CpG3 (TTGAGTGGGTGTGAATTTTAT) were designed to be (5'->3') forward (Table 3).

For data quality assurance, only samples exhibiting a standard deviation of less than 3% in DNA methylation between technical replicates were selected for further analysis. This minimizes experimental variability and enhances the reliability of the results obtained. Furthermore, the pyrosequencing analysis included DNA samples with known methylation levels (0%, 25%, 50%, 75%,

and 100%) as controls (EpiTect Control DNA, Qiagen) to identify potential biases from differential amplification of methylated fragments. The reference controls enabled the evaluation of the accuracy and consistency of the experimental procedures.

To mitigate the influence of batch effects, all steps involved in DNA methylation analysis, including bisulfite conversion, PCR, and pyrosequencing, were scrupulously processed using a balanced design approach.

Table 3 **Primer Sequences (5'->3')** for Pyrosequencing targeting CpG Sites in the experiment

	Primer Seq (5'->3')
Pyrosequencing for Cpg1,2	GAGGATAAAAGATAGTGTTTTAGTT
Pyrosequencing for Cpg3	TTGAGTGGGTGTGAATTTTAT

2.5 Statistical analyses

The statistical tests and visualizations were conducted using R version 2023.6.1.524, using the RStudio interface (Team, 2023). The primary R packages applied included xtable (Dahl et al., 2019), dplyr (Wickham et al., 2023), tidyr (Hadley Wickham, 2023), and ggplot2 (Wickham, 2016). A fully annotated version of the analysis code is available from the authors upon reasonable request. In general values were rounded to two decimal places, and significance was determined at a threshold of $p < 0.05$.

The correlation analysis was conducted for the purpose of evaluating whether DNA methylation levels of individual CpG sites are associated with each other.

To comprehensively understand the dataset, descriptive statistics were generated, including the mean, SD, minimum, maximum, median, and interquartile range (Q25-Q75). Furthermore, a box plot was generated to visualize the distribution of DNA methylation levels.

To assess the interaction between three factors Status (BED+/-), Sex (female/male), and Position (CpG1, CpG2, CpG3 and mean CpG)) and their impact on mean DNA methylation percentage a Three-Way ANOVA without repeated measures was calculated. When a significant interaction was identified, post-hoc pairwise comparisons were conducted to determine specific group differences.

Pearson correlations were performed to assess the relationship between DNA methylation levels and BMI (hypothesis driven) and EDE-Q scores (exploratory approach).

3 Results

In this section, the results of the thesis are presented. First, the cohort is characterized to provide the groundwork for subsequent analyses. Next, correlation analyses are reported, serving as an important basis for the following steps. To provide an overview of the data distribution for DNA methylation levels in different CpG positions a general Boxplot is shown. Afterward, each of the hypotheses is addressed individually. Moreover, a preliminary data set is shown.

3.1 Cohort under examination

The characteristics of the groups is presented in Table 4 and Figure 5. A chi-square test was conducted to compare the distribution of sex between the BED- and BED+ groups. The chi-square test showed that there was no significant difference in sex distribution between the groups. The individual t-test was used to compare BMI and age. There was no significant difference in age nor BMI indicating that the groups were well matched on these demographic variables

In contrast, the BED+ group scored significantly higher on the EDE-Q total score, reflecting greater overall eating disorder psychopathology between the groups. Subscale analyses showed significantly elevated scores in Eating Concern, Weight Concern and Shape Concern among BED+ participants. No significant difference emerged for the Restraint subscale.

In general, the examined cohort is suitable for comparison between the groups. There was no significant difference for the categories of sex, age, and BMI. This indicates that the groups are well matched and that these factors are unlikely to have caused differences between the groups.

They differ significantly in EDE-Q scores, which distinguishes BED + from BED. For the reader's convenience, the summarized data are presented in Table 4 and Figure 5.

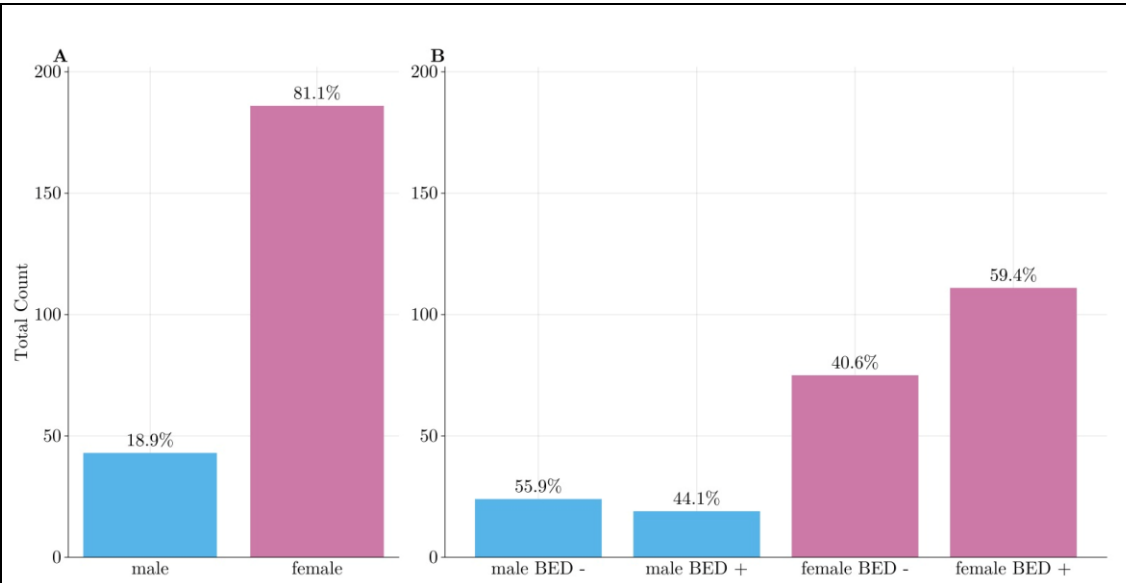


Figure 5 A: Distribution of participants by sex

B: Distribution of participants by sex and in BED+ and BED- status

The bar chart shows the total number and percentage of male and female participants in BED+/- groups.

Table 4 Characteristics of the cohort

The characteristics of the cohort, including the group with BED+ and BED-, are presented. The variables that were examined comprise the distribution of sex, BMI, age, scores of EDE- Q, and testing for normal distribution.

Status	BED -	BED +	Group Differences
N	99	130	
Female/male %	75.8/24.2	84.6/14.6	Chi-Square= 2.72 p= 0.1
BMI (Mean/SD)	38.24/6.5	38.56/7.82	T= -1.12 p= 0.27
Age (yrs, Mean/SD)	39.5 /13.6	41/12.6	T = - 0.81 p= 0.42
Age (yrs), range	19-73	19-67	-
EDE Q total score (Mean/SD)	1.95/1.21	2.71/0.87	T= -3.93 p<0.01
EDE-Q Restraint (Mean/SD)	1.52/1.47	1.77/1.31	T= -1.01 p= 0.32
EDE-Q Eating Concern (Mean/SD)	1.52/1.16	2.27/1.34	T= -3.6 p <0.01
EDE-Q Weight concern (Mean/SD)	1.82/1.91	3/1.45	T= -3.84 p<0.01
EDE-Q Shape concern (Mean/SD)	2.94/1.8	3.81/1.21	T= -3.05 p<0.01

3.2 Correlation Analysis of DNA methylation Levels between individual CpG Sites 1-3

The correlation analysis presented in Table 5 was conducted for the purpose of evaluating whether DNA methylation levels of individual CpG sites are correlated with each other. The outcomes of the analysis indicate significant positive correlations between all sites. In such instances, the mean DNA methylation level of the correlated CpG sites is standard practice and reported in the further investigation (Eulalio et al., 2025, Giel et al., 2022b, Su et al., 2013).

Table 5 Correlation Analysis of DNA methylation Levels between individual CpG Sites 1-3
This table shows the correlations of DNA methylation levels between different CpG sites (CpG1, CpG2, and CpG3). It includes the correlation coefficients, significance levels, p-values, and 95% confidence intervals (CI) for each pairwise comparison.

Variable 1	Variable 2	Correlation	Significance	p- value	95% CI (Lower)	95% CI (Upper)
CpG1	CpG2	0.18	**	< 0.01	0.05	0.31
CpG1	CpG3	0.23	***	< 0.01	0.11	0.35
CpG2	CpG3	0.26	**	< 0.01	0.13	0.38

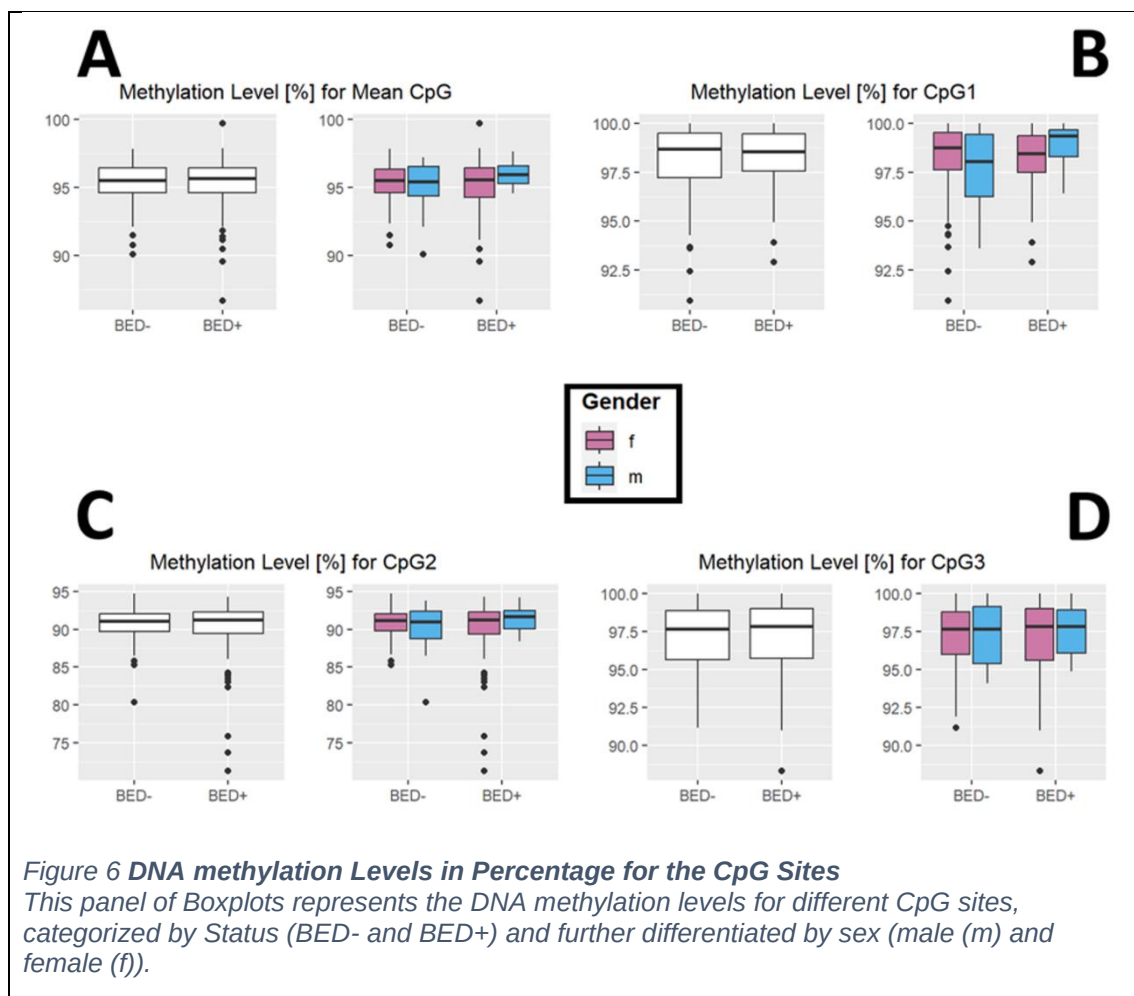
3.3 Boxplot Visualization of CpG methylation Levels in the Total Sample and by Sex

The purpose of this boxplot is to provide an overview of the data distribution. The DNA methylation levels for the mean CpG and individual CpG sites are presented in Figure 6. Mean DNA methylation percentages were generally high (>90%) across BED- and BED+ groups, with similar median values and overlapping interquartile ranges. Among the sites, CpG2 exhibited the lowest DNA methylation levels, while CpG1 showed the highest median.

Stratification by sex revealed generally similar DNA methylation means between males and females. At CpG1, males with BED+ had the highest observed

difference in DNA methylation, with a mean of 98.9% (SD = 1.10) compared to 97.7% (SD = 1.87) in BED- males therefore representing a 1.2% difference. CpG2 exhibited a smaller difference in mean value of 0.9%, while CpG3 showed a minimal difference. No comparable differences were observed in females. The significance of these group differences were later assessed using ANOVA.

The precise numerical values can be located in the *Supplemental Data*.



3.4 H1: DNA methylation levels of the NOP receptor gene differ between individuals diagnosed with BED and BMI-matched control participants without BED

To address the first hypothesis, it was examined whether the BED status (BED+ vs. BED-) was associated with differences in DNA methylation at the three examined CpG sites within NOP. The results of the ANOVA showed no significant main effect of BED status on DNA methylation levels at NOP ($F(1,890) = 0.12, p = 0.73$). Detailed values can be found in Table 6.

3.5 H2: Sex-specific differences exist in DNA methylation levels of the NOP receptor gene among individuals with BED and BMI-matched controls

The ANOVA showed no significant main effect of sex on DNA methylation levels ($F(1,890)=1.34, p=0.25$) indicating no overall significant difference between males and females regardless of BED status. However, there was an interaction effect between BED status and sex that was statistically significant ($F(1,890)=7.6, p<0.01$). Because of this result, a post-hoc pairwise test was conducted (Table 6, Table 7). For males, the comparison between these two groups produced a statistically significant difference (estimate = -0.776, $p=0.02$), indicating that DNA methylation levels are significantly higher in males with BED compared to males without BED. This difference was not statistically significant (estimate = 0.264, $p=0.11$) between BED- and BED+ females.

Table 6 Three-Way ANOVA Analysis for Status (BED+/-), Sex(male/female), and Position (CPG1,2,3, mean of the regions) and their interaction with DNA methylation levels in [%]

	<u>Df</u>	<u>Sum Sq</u>	<u>Mean Sq</u>	F-Value	p-Value	
Status	1	1	0.6	0.12	0.73	
Sex	1	6	6.5	1.34	0.25	
Position	3	8005	2668.5	554.05	> 0.01	***
Status: Sex	1	37	36.6	7.6	> 0.01	***
Status: Position	3	11	3.8	0.78	0.50	
Sex: Position	3	1	0.4	0.09	0.97	
Status: Sex: Position	3	7	2.4	0.5	0.68	
Residuals	890	4286	4.8			

Table 7 Post Hoc Test Pairwise Comparisons for Status x Sex Interaction on DNA methylation Levels (estimated marginal means (EMMEAN))

Status	Sex	EMMEAN	SE	DF	Lower CL	Upper CL
BED-	M	95.13	0.22	894	94.69	95.57
BED+	M	95.91	0.25	894	95.41	96.40
BED-	F	95.40	0.13	894	95.15	95.65
BED+	F	95.14	0.10	894	94.93	95.34

Pairwise Comparison by Sex

<u>Sex</u>	<u>Contrast</u>	<u>Estimate</u>	SE	DF	t-ratio	<u>p-value</u>
male	BED- – BED+	-0.776	0.34	894	-2.3	0.02*
female	BED- – BED+	0.264	0.16	894	1.61	0.11

3.6 H3: BMI Scores correlate with DNA methylation levels of the NOP receptor gene

The correlation coefficients and corresponding p-values for BMI in relation to the mean CpG methylation (mean of CpG1-3) was calculated. No significant correlation was found, neither for the total sample nor for one of the respective subgroups separated by BED status (Table 8).

Table 8 Correlation Analysis between Mean CpG Levels and BMI

The table presents the correlation coefficients and corresponding p-values for BMI in relation to the mean CpG methylation (mean of CpG1-3) across the entire cohort. The cohort was then stratified into BED+ and BED- groups for further analysis

Mean CpG (CpG1-3) &	Correlation coefficient (r)	p-Value
All subjects		
BMI	0.08	0.22
Individuals with BED +		
BMI	0.1	0.24
Individuals without BED -		
BMI	0.04	0.7

3.7 Results of exploratory data analysis

This section presents the findings of an exploratory analysis conducted to investigate potential patterns and relationships within the dataset. It is important to note that this analysis was conducted without a predefined hypothesis or existing literature to guide the inquiry.

3.7.1 Exploratory ANOVA: Interaction effects of BED Status, Sex, and CpG Position on DNA methylation

The collected dataset was analysed in a three-way ANOVA to explore the impact of three factors: BED status, sex, and position on mean DNA methylation levels expressed as percentages. While part of the analyses were hypothesis-driven, the results presented here concern exploratory, descriptive

analyses of residual data for which no specific hypotheses had been formulated.

A significant main effect was found for position ($F(3,890)=554.05$, $p<0.01$), indicating differences in DNA methylation levels across CpG sites.

Regarding interactions no significant interactions were found for BED Status $\times$ Position ($F(3,890)=0.78$, $p=0.50$), Sex $\times$ Position ($F(3,890)=0.09$, $p=0.97$), or BED Status $\times$ Sex $\times$ Position ($F(3,890)=0.5$, $p=0.68$). Full results are presented in Table 6.

3.7.2 EDE-Q Scores and their correlation with DNA methylation levels for the NOP receptor gene

In this exploratory study, the correlation coefficients and corresponding p-values for the relationship between mean CpG methylation and the total score of the EDE-Q, as well as its subcategories, were calculated. This was undertaken for the entire cohort, as well as for individuals with BED and those without BED.

Across the entire cohort without separation by BED status, no significant correlation was found between mean CpG methylation and EDE-Q total score ($r=0.10$, $p=0.22$). A weak but statistically significant positive correlation was observed for the Restraint subscale ($r=0.17$, $p=0.03$). Associations with Eating Concern, Weight Concern, and Shape Concern were all non-significant.

When separated by status the BED+ group, had no significant correlations detected between mean CpG methylation and total or subscale scores.

In contrast, among individuals without BED, significant positive correlations were noted between mean CpG methylation and EDE-Q total score ($r=0.34$, $p=0.02$), Restraint ($r=0.39$, $p=0.01$), and Shape Concern ($r=0.32$, $p=0.02$), while Eating Concern and Weight Concern showed no significant relationships (Table 9).

Table 9 Correlation Analysis between Mean CpG Levels and eating disorder pathology as assessed by EDE-Q

The panel shows the correlation coefficients and associated p-values with the mean CpG methylation (mean of CpG1-3) and the Total score for the Eating Disorder Examination Questionnaire, as well as its subscales; Restraint, Eating Concern, Weight Concern, and Shape Concern. The entire cohort was subjected to evaluation, after which the two groups were divided into BED+ and BED- categories for testing.

Mean CpG (CpG1-3) &	Correlation coefficient (r)	p-Value
All subjects with no difference between BED+/-		
EDE Q total score	0.1	0.22
Restraint	0.17	0.03*
Eating Concern	-0.03	0.69
Weight concern	0.1	0.24
Shape concern	0.05	0.5
Individuals with BED +		
EDE Q total score	0.01	0.9
Restraint	0.1	0.32
Eating Concern	-0.06	0.51
Weight concern	0.07	0.44
Shape concern	-0.06	0.53
Individuals without BED -		
EDE Q total score	0.34	0.02*
Restraint	0.39	0.01**
Eating Concern	0.1	0.48
Weight concern	0.2	0.16
Shape concern	0.32	0.02*

4 Discussion

This thesis investigated the association of epigenetic changes in NOP in the obesity spectrum. Therefore, the potential of measurable changes in DNA methylation at NOP between individuals with BED and those with obesity but without BED were explored. Furthermore, it examined whether these changes could offer insights into potential sex differences. In order to address this question, DNA methylation levels were assessed at three CpG sites located within the first intron of the NOP gene. A descriptive analysis characterised the sample in terms of age, sex, BMI, and eating disorder psychopathology (measured by EDE-Q). Furthermore, correlation analyses were conducted to examine possible relationships between DNA methylation at the CpG sites and clinical variables such as BMI and EDE-Q.

In this section, the results of this thesis are deliberated and put into the context of the body of literature. Additionally, general strengths, limitations, and future perspectives are discussed. An outlook on DNA in clinical practice is also provided.

4.1 H1: DNA methylation levels of the NOP receptor gene differ between individuals diagnosed with BED and BMI-matched control participants without BED

The first hypothesis assumed that there is a difference in DNA methylation levels between individuals with BED and a BMI-matched group without BED in NOP. The results do not support this hypothesis. There was no significant main effect in DNA methylation levels between the groups suggesting that DNA methylation at this locus does not differ between individuals with BED and BMI-matched controls.

This study did not investigate gene expression. Whereas these findings suggest that NOP DNA methylation may not differentiate between groups, differences in gene expression could still exist and may be mediated by mechanisms other than DNA methylation. Such changes are sometimes driven by a single epigenetic mechanism. For instance, prior work has shown selective

modulation via histone acetylation without parallel changes in DNA methylation or other epigenetic marks (Pucci et al., 2019).

Research specifically on histone modifications regulating NOP expression in the context of BED is limited (Caputi et al., 2019). Nevertheless, studies on other opioid receptors (mu, delta, kappa), which belong to the same receptor family as NOP (Donica et al., 2013), have demonstrated that histone modifications can play a critical role in regulating gene expression, particularly in the context of drug abuse. In some cases, these changes have been found to be reversible (Reid et al., 2022). Moreover, it has been shown that eating behaviour and food restriction can influence NOP mRNA expression (Pucci et al., 2016, Rodi et al., 2002), suggesting that the dysregulated feeding patterns characteristic of BED may impact NOP regulation.

While no DNA methylation differences were found at the studied CpG sites, which are located within the first intron of the NOP gene, it remains possible that other regions of the gene are affected.

Introns, which used to be considered to be non-coding sequences, are getting recognised as playing a significant regulatory role. Specifically, promoter-proximal introns, can enhance gene expression by increasing mRNA accumulation, influencing transcription initiation and improving transcript stability and nuclear export (Dwyer et al., 2021, Rose, 2018, Shaul, 2017). The intron investigated in this thesis could be considered a promoter-proximal intron. However, it remains unclear whether such regulatory mechanisms apply to the studied gene, as evidence for intron-mediated regulation for the gene is not demonstrated yet.

Studies on other opioid receptor genes, including the mu, delta and kappa subtypes, have revealed that intronic regions, particularly the first intron, play a significant role in transcriptional regulation by interacting with specific regulatory factors (Wei and Loh, 2011, Lu et al., 2014). Within this framework, it is possible that epigenetic modifications within the first intron of NOP may have an influence for transcriptional control and functional outcomes.

On the other hand, promoter hypermethylation has repeatedly been linked to opioid use, both therapeutic and abusive, as well as to short- and long-term

exposure (Chorbov et al., 2011, Sandoval-Sierra et al., 2020, Tang et al., 2023, Zhang et al., 2022) and altered sensitivity to morphine analgesia (Zhou et al., 2014). DNA methylation changes in promotor regions have also been observed in the context of neuropathic pain (Jiang et al., 2022). Furthermore, emerging evidence indicates differential DNA methylation of the kappa opioid receptor gene in individuals with borderline personality disorder (Gescher et al., 2024). Regarding the NOP receptor, differences in promoter methylation have been reported in Alzheimer's disease, correlating with decreased gene expression (Xu et al., 2018). Emerging data implicate NOP DNA methylation changes in the promotor can be also be associated with Alcohol Dependence (Zhao et al., 2022).

Although promoter regions are the most extensively studied and recognised sites for epigenetic regulation, additional gene regions, including introns, exons and distal enhancers, may also contribute to the regulation of opioid receptor expression and therefore, all these regions might be affected by DNA methylation changes.

4.2 H2: Sex-specific differences exist in DNA methylation levels of the NOP receptor gene among individuals with BED and BMI-matched controls

The hypothesis assumed that there is a sex-specific difference in DNA methylation levels between individuals with BED and a BMI-matched group without BED in NOP. ANOVA analysis revealed a significant interaction between BED status and sex, suggesting that BED status influences DNA methylation levels differently in males and females. A more in-depth examination of the mean values at individual CpG sites provides further insight into this pattern. At CpG1, BED+ males exhibited higher DNA methylation compared to BED- males, whereas no meaningful difference was observed in females. Post hoc pairwise comparisons confirmed that this difference was significant in males but not in females, supporting the conclusion that the observed DNA methylation changes in BED might be driven by sex-specific effects.

This finding suggests that the differences in DNA methylation observed in males with BED compared to their BMI- matched group are not consistent across the analysed CpG sites but appear to be specific to one site. CpG1 exhibited the most noticeable difference, whereas the effect was more moderate at CpG2 and only minimal at CpG3. This pattern indicates that the DNA methylation differences are not uniform across sites. The interpretation of a site-specific effect is further supported by the non-significant interaction between BED status and position, showing that BED-related DNA methylation differences were not statistically stronger at one CpG site compared to another. Similarly, the three-way interaction between BED status, sex, and position was non-significant, suggesting that the pattern of BED-related DNA methylation differences across CpG sites was comparable for males and females. Collectively, these results support the conclusion that the observed DNA methylation effect is limited to one site rather than reflecting a consistent or sex-dependent pattern across all investigated CpG sites. While these changes may appear negligible, it is increasingly recognised that alterations in methylation of less than 10% can still be functionally relevant and may contribute to disease development (Leenen et al., 2016).

Overall, these results suggest that the difference observed between the male groups (BED+/BED-) at CpG1 may be a localised effect. Given that this result emerged at only one of three examined CpG sites and was limited to males, the finding could represent a statistical artefact. In epigenetic association studies, the risk of type I error is heightened due to multiple testing and sample size limitations, which raises the possibility that such isolated results occur by chance rather than reflecting meaningful biological differences (Manu et al., 2022, Riancho et al., 2016).

These findings are partly consistent with previous research demonstrating sex-specific differences in DNA methylation. Sex differences in DNA methylation are widespread and complex, affecting many CpG sites, including autosomes and sex chromosomes, varying with age and disease context (El-Maarri et al., 2007; Reiner et al., 2023; Singmann et al., 2015; Watanabe et al., 2016; Yusipov et al., 2020). Prior studies have identified numerous CpG sites exhibiting

differential methylation between males and females, suggesting a broader epigenetic basis for sex differences in various biological processes.

Furthermore, previous studies have reported inconsistent findings regarding sex differences in DNA methylation, with some reporting slightly higher methylation in males at most CpG sites (El-Maarri et al., 2007) and others observing higher levels in females (Reiner et al., 2023). In the present study, no significant main effect of sex on methylation was detected.

Currently, there are no published studies directly examining sex-related DNA methylation differences for NOP. Nevertheless, studies on other opioid receptors, have reported sex-specific DNA methylation patterns associated with differences in opioid use disorder and analgesic response (Agulló et al., 2023). These findings suggest that, in this cohort, sex alone did not lead to differences in NOP receptor gene DNA methylation. This underscores the importance of considering additional factors, such as BED status. However when classified by BED status, a sex-specific difference emerged, which aligns with prior research identifying sex-specific epigenetic changes in mental health (Tesfaye et al., 2024) and BED contexts (Giel et al., 2022b).

In summary, the results of this study provide partial support for the hypothesis that DNA methylation levels differ between males when grouped according to BED status with the limitation of this effect might be site-specific rather than widespread.

4.3 H3: BMI Scores correlate with DNA methylation levels of the NOP receptor gene

Hypothesis 3 was not supported. No statistically significant correlations were found between BMI scores and NOP receptor gene DNA methylation levels, either in the full cohort or when examined by BED status. This finding indicates that any observed relationship between BMI and CpG methylation is weak and probably irrelevant for this gene.

While earlier studies have identified significant associations between BMI and DNA methylation at specific CpG sites (Wahl et al., 2017), other research has

failed to find a strong or consistent link across all sites (Giel et al., 2022b, Rodríguez-López et al., 2021). To date, no studies have specifically examined the impact of BMI on DNA methylation on NOP. The present findings therefore add to the existing evidence that points to a lack of a direct or uniform link between BMI and DNA methylation.

4.4 Discussion of exploratory data analyses

All of the following results should be interpreted with caution given the exploratory nature of the approach.

4.4.1 Exploratory ANOVA: Interaction effects of BED Status, Sex, and CpG Position on DNA methylation

Exploratory analyses were conducted on the remaining data to examine potential interactions between BED status, sex, and CpG position on DNA methylation.

The main effect of position was highly significant, indicating that DNA methylation levels differ across CpG sites. In general, CpG sites in close proximity exhibit similar methylation patterns because the enzymatic machinery responsible for adding or removing methyl groups usually affects neighbouring CpGs in a similar way (Affinito et al., 2020). In the present study, the examined gene region is highly methylated across all three sites. Although CpG2 exhibits lower DNA methylation than CpG1 and CpG3, the absolute difference is modest. This suggests that the lower methylation at CpG2 may not have a significant functional impact, despite being statistically different from neighbouring sites. Still it could also be possible that the observed site-specific difference reflects the influence of local regulatory features, including chromatin accessibility, transcription factor occupancy, or other epigenetic elements specific to the locus (Tibben and Rothbart, 2024).

For the interaction between sex and position, there is no significant effect. This suggests that the DNA methylation levels across different positions do not vary significantly between males and females.

In the present study, no significant interactions were found between BED status and position. This suggests that differences in DNA methylation across the three examined CpG positions are not dependent on BED status. Therefore, BED status alone does not lead to differential methylation across the studied positions, and the methylation pattern is generally similar across positions, regardless of BED status.

Similarly, the three-way interaction between BED status, sex and position was also non-significant. This indicates that the combined influence of BED status and biological sex on DNA methylation does not differ across the CpG positions, so even when considering both BED status and sex together, there is no evidence that certain positions are more or less affected by these factors.

In summary, the interaction between sex and position indicates that DNA methylation levels do not differ significantly between males and females across different CpG positions. However, the main effect of position is suggesting that DNA methylation levels vary considerably across the different CpG positions, with CpG2 being markedly lower than the other positions. Additionally, the three-way interaction among BED Status, sex, and position suggests that the combined effect of these factors does not significantly influence DNA methylation levels.

Taking all of this into account, the observed differences are more likely to be limited to specific CpG sites than to reflect systematic patterns across all positions, groups, and subgroups.

4.4.2 EDE-Q Scores and their correlation with DNA methylation levels for the NOP receptor gene

In the total sample, Restraint scores demonstrated a weak but statistically significant positive correlation with mean CpG methylation. No significant correlations were observed for the other EDE-Q categories.

Among individuals with BED, none of the correlations achieved statistical significance, indicating an absence of association between CpG methylation and self-reporting scores in this group.

By contrast, in individuals without BED, the total EDE-Q score demonstrated a moderate correlation indicating statistical significance. The Subcategory Restraint scores showed a moderate and statistically significant positive correlation with CpG methylation. Additionally, Shape Concern demonstrated a weaker yet still significant correlation.

These findings indicate that high total EDE-Q scores as well as restraint and shape concern scores might be linked to increased CpG methylation in people without BED. This association was not observed in individuals diagnosed with BED.

4.5 Strengths and Limitations

In this section, the study's strengths and limitations are discussed, covering aspects of cohort characteristics, diagnostic criteria, the selection of single-gene and CpG sites, laboratory workflow, and statistical analyses.

The study's large sample size ($N > 200$) was moderate and well characterized in BED+ and BED- through the examination of the EDE-Q score and its subcategories. The samples were age- and BMI-matched to ensure comparability between the BED+ and BMI- groups.

The sample exhibited a considerable sex imbalance, with 81% of subjects being female and 19% male, which could potentially influence analyses that include sex as a factor. An unequal representation of sexes may affect the estimation of sex-specific effects and reduce the generalizability of the findings to population with equal representation of males and females. Nevertheless, across all CpG sites, the DNA methylation values of females and males within the BED+ and BED- groups largely overlapped. Therefore, while the imbalance should be considered a limitation, it likely did not substantially influence the group-level comparisons in this

study.

The relatively small size of the male subgroup may limit the reliability of comparisons between BED+ and BED– males. As the influence of individual data increases, it is possible that observed effects may be attributed to individual variability rather than to generalisable patterns (Faber and Fonseca, 2014, Pocock et al., 2002). The relatively low number of males in the BED+ group (N=19) and the BED– group (N=24) may limit the statistical power for detecting differences between the two groups. This small male sample size may make the analysis more vulnerable to random variations, potentially reducing the reliability of the results for the male subgroup. Importantly, descriptive data showed that individual DNA methylation values were tightly clustered within both male groups. This suggests that the influence of any individual participant may not be disproportionate.

Furthermore, this cohort has been previously tested (Giel et al., 2022b), focusing on a different gene, but also reporting effects specifically in the male BED+ versus BED– comparison. As this represents only the second study to report this effect in this cohort, the possibility of selection bias is worth considering, though it does not substantially undermine the current findings.

A number of factors may contribute to variations in DNA methylation levels, which could act as potential confounding variable in this study. In particular, the study did not control for potential confounding factors, including medication (Toth, 2021, Zhou et al., 2021), smoking (Lee and Pausova, 2013), diet as well as nutritional values (Amenyah et al., 2020b; McGarel et al., 2015; Steiger and Booij, 2020) and socioeconomic status (Tehranifar et al., 2013), all of which could influence DNA methylation levels.

Furthermore, as stated in the introduction, BED is associated with a high prevalence of other health comorbidities (Kessler et al., 2013, Mars et al., 2025). This study did not assess comorbidities, particularly mental health. This represents potential confounding bias (Skelly et al., 2012).

It is noteworthy that the mean BMI for both groups was approximately 38, which is considerably higher than the normal BMI range of 18.5–24.9. Although no general studies have explicitly reported the average BMI in individuals with BED, one investigation using data from the Collaborative Psychiatric Epidemiologic Surveys (N = 14,497) examined participants with bingeing and BED. In this sample, the mean BMI was 27 (Brown et al., 2018). A BMI of 38 indicates roughly 40% more body weight for the same height than a BMI of 27. Furthermore, the study specifically targeted individuals actively seeking treatment, which offers insights into a clinically relevant population.

The high mean BMI and the focus on a treatment-seeking population, suggests a more severe case profile and therefore potentially excluding populations with less symptoms or lower levels of distress.

Despite these limitations, the study's design in a BMI and aged matched approach contributes valuable data to the field, laying a foundation for future work. This is especially important given the lack of research conducted in the field of epigenetics in BED in general (Hubel et al., 2019).

In this study, BED diagnoses were based on DSM-IV (American Psychiatric Association, 1994) criteria for consistency, providing a stricter approach than that taken in the newer DSM-5 (American Psychiatric Association, 2013), particularly in terms of the more conservative time criteria (Vo et al., 2017). But for the future DSM-IV criteria may restrict the comparability of results with those of more recent studies that apply DSM-5 standards.

Additionally, the EDE-Q Questioner was applied. As a reliable self-report instrument, the EDE-Q is well-suited for assessing the specific psychopathology of BED and offer a number of advantages like cost-effectiveness and standardisation (Hilbert and Tuschen-Caffier, 2016). Nevertheless, self-report questionnaires also present a number of challenges, for example including the desire for social acceptance, response bias, variability in self-awareness and the subjective interpretation of questions (Demetriou et al., 2015). It also should be noted that some missing data on self-report has occurred in this study.

This study adopts a single-gene approach as opposed to a whole-genome analysis. This approach is cost- and time-efficient, as it simplifies both the workup and the data analysis. Additionally, focusing on specific CpG sites within a gene allows for a detailed examination, which could uncover epigenetic modifications that might be overlooked in broader analyses.

On the other hand, this strategy limits the ability to draw conclusions about the broader epigenetic landscape in individuals with BED. Moreover, it carries the risk of overlooking complex gene–gene interactions and coordinated regulation across multiple loci. Because the broader genomic context is not considered, these potential network-level effects remain unexplored. In this respect, a genome-wide approach could provide a more integrated view of the molecular mechanisms underlying BED.

When this thesis was developed, genome-wide analyses specifically targeting BED had not been performed.

Since then, progress has been made through a landmark genome-wide association study, which identified several risk loci near the HFE, MCHR2, and LRP11 genes. The findings suggest a shared basis between BED and certain neuropsychiatric traits, while also highlighting iron metabolism as a biological process that may play a role in the development of BED (Burstein et al., 2023). More recently, a large-scale genomic meta-analysis has extended these findings, detecting additional loci associated with binge eating behaviour and revealing shared and distinct genetic correlations with AN (Termorshuizen et al., 2025). Although this study has not yet undergone peer review, together these studies highlight the growing feasibility and potential of genome-wide approaches in revealing the complex polygenic basis of BED.

It is important to recognise that much of the pharmacological evidence originates from studies using non-selective opioid receptor antagonists, such as naloxone (Drewnowski et al., 1995, Grilo et al., 2021, Grilo et al., 2022). These compounds

target the classical mu, delta and kappa opioid receptors, and have a weaker interaction with the NOP receptor (Spagnolo et al., 2008). Therefore, behavioural effects observed in the human trials, such as reduced food intake or altered reward responses, may reflect a more general opioid receptor blockade rather than specific NOP-mediated mechanisms. This overlap limits the ability to attribute findings from these pharmacological studies to NOP.

To this date, no studies have examined the effects of selective NOP receptor agonists or antagonists on eating behaviour in humans. However, this has been done in animal models. In rats the intracerebroventricular administration of specific NOP receptor agonists induced marked hyperphagia, whereas specific NOP antagonists effectively blocked this effect (Economidou et al., 2006). These findings suggest that the selective activation of NOP receptors, independently of other opioid receptors, can modulate feeding behaviour. Whether this can be translated to humans remains to be determined.

The thesis focuses on specific CpG sites identified by prior research (Ruggeri et al., 2015, Ruggeri et al., 2018). These sites were derived from a genome-wide methylation analysis and subsequently investigated in a hypothesis-driven approach in a later paper. However, inconsistencies exist between these publications. While the initial study identified three methylation sites, the follow-up study examined only two of these CpG sites. The rationale for this selection was not explicitly stated in the follow-up paper, nor was it clear which or why one of the original three sites was excluded from further investigation.

To address this gap, the present thesis examines all three CpG sites identified in the genome-wide findings. Additionally, it is important to note that papers focused on a different disease pattern. Although the term binge is used for both BED and binge drinking, suggesting a close resemblance, the two share certain characteristics but remain nevertheless different disorders (Escrivá-Martínez et al., 2020). Individuals with higher impulsivity, sensitivity to reward, or personality traits such as neuroticism and sensation-seeking may be more vulnerable to both behaviours (Ferriter and Ray, 2011, Van Swearingen and Noel, 2024), suggesting a possible shared psychological and neurobiological basis underlying their

development. Both bingeing behaviours are often used as a way of coping with stress or negative feelings (Freeman and Gil, 2004; Grzywacz and Almeida, 2008; Li et al., 2024; Vornlocher and Shiota, 2024). Furthermore, the co-occurrence of risky drinking and binge eating has been widely described in the literature (Bogusz et al., 2021, Friedman et al., 2023).

However, it is unclear whether these two behaviours influence each other. Inconsistent results have been found in studies exploring the directionality between BED and binge drinking. Some evidence suggests that eating pathology may precede or exacerbate alcohol misuse, possibly serving as a coping mechanism for guilt or negative emotions (Field et al., 2012, Measelle et al., 2006). Findings have shown that the presence of an eating pathology can predict future increases in substance use, whereas the reverse relationship is not evident (Measelle et al., 2006). Another study showed that binge drinking significantly increased the likelihood of binge eating later on in the same day, suggesting that alcohol consumption may acutely trigger binge eating episodes (Fischer et al., 2023). However, other studies have failed to confirm this pattern, indicating that the association may be influenced by multiple factors, bidirectional dynamics, or context-dependent variables (Micali et al., 2015, Sonnevile et al., 2013).

BED and binge drinking also differ in terms of motivation and context. BED episodes are often solitary and secretive and are accompanied by shame (Giel et al., 2022a). In contrast, binge drinking episodes are usually socially reinforced and often occur in social contexts such as university drinking culture. They can be perceived as normal or even encouraged (Ross-Houle and Quigg, 2019). This difference suggests that BED might be more inwardly driven, while binge drinking may be influenced more often by external social factors or reinforcement.

Another fundamental difference lies in the biological necessity of the substance involved. Food is essential for survival, so complete abstinence is neither possible nor desirable. Alcohol, however, is a psychoactive and inherently toxic substance, and treatment for binge drinking and alcohol use disorder emphasises reduction or

cessation (Akbar et al., 2018). Furthermore, alcohol withdrawal is well documented (American Psychiatric Association, 2013, Schulte et al., 2016), whereas withdrawal symptoms related to food are neither clearly defined nor clinically established (Escrivá-Martínez et al., 2020, Schulte et al., 2018).

Taken together, these differences suggest that, despite the presence of overlapping features between BED and binge drinking, their distinct physiological and psychosocial foundations may act as constraints on the translation of findings from one domain to the other. Still these CpG sites represent a promised target for investigation within the framework of this thesis since there is limited research done in epigenetic of eating disorders in general and more specific, to our knowledge, no prior studies have examined NOP in context of DNA methylation in relation to any eating disorder, underscoring the significance of this work.

The study's statistical method provides a solid framework for analysing methylation patterns and their associations with BED-related variables, but limitations must be acknowledged.

The combination of descriptive statistics, correlation analysis, and three-way ANOVA allows for a thorough analysis of DNA methylation differences, with the three-way ANOVA particularly useful for its simultaneous examination of factors (BED Status, Sex, and Position) and their interaction. However, interpreting interactions between multiple factors can be challenging, especially when higher-order interactions (BED Status $\times$ Sex $\times$ Position) are involved.

The mean DNA methylation level of correlated CpG sites is a common epigenetics practice. This approach simplifies the analysis, producing clearer conclusions.

While this improves interpretability, it may also mask effects. Individual CpG sites may exhibit distinct DNA methylation patterns that are lost when averaged, potentially reducing resolution. To address this, DNA methylation levels at all CpG sites were also examined individually, allowing for the detection of site-specific differences.

The distribution of mean DNA methylation levels in this study reveals a dynamic range, with no markedly low values and an upper boundary at 100 %. Because

DNA methylation is measured as a proportion of methylated cytosines, values are mathematically constrained between 0 and 100 %, meaning that very high DNA methylation levels cannot exceed this limit. As a result, differences between highly DNA methylated sites are compressed, reducing the numerical differentiation that can be observed. Given the consistently high levels of DNA methylation for most individuals across all CpG sites, particularly at CpG 1 and CpG 3, the data suggest a potential ceiling effect. This clustering near the upper limit may potentially limit meaningful variations and limit the sensitivity of statistical analyses. This upper-range clustering may limit the capacity to detect subtle variations or limit the sensitivity of statistical analyses.

The laboratory methods used are well-established and commonly employed to answer this type of research question. Quality checks were conducted at several stages of the workup, as outlined in the materials and methods.

To minimize technical variability, DNA concentrations were standardized across samples, bisulfite conversion was verified via PCR amplification, negative PCR controls were included, technical replicates were performed with a maximum standard deviation of 3%, and reference DNA controls with known DNA methylation levels were used.

It is to note, that not all analyses were performed by a single researcher. The DNA isolation and bisulfited conversion was carried out by a different individual who previously worked on the same sample set but with a different research question. Importantly, because the same person handled all samples, any potential handling error would likely affect all samples equally, making it less likely to systematically bias group differences and introducing a systematic rather than differential bias.

The present study concentrates exclusively on DNA methylation; consequently, the potential involvement of other epigenetic mechanisms, such as histone modifications or non-coding RNA regulation, remains to be assessed. Therefore, the possibility that these additional processes play a role in gene regulation and the disorder cannot be ruled out.

Also Genotyping was not incorporated in this study, despite the recognised influence of DNA sequence has a direct effect on DNA methylation patterns (Deaton and Bird, 2011; Lienert et al., 2011; O'Donnell and Meaney, 2020). Without accounting for these genetic differences, the results could be influenced by underlying genetic variability, affecting the interpretability and generalisability of the findings.

A cross-sectional study design was selected for this investigation. The advantages of this approach include its time efficiency and simplified data collection process, since it does not require follow-up, thereby enabling larger sample sizes and as consequence enhances the statistical power. On the downside the risk of confounding is increased, as this design captures data at a single time point, making it challenging to account for all potential influencing factors. On top of that, cross-sectional studies cannot establish causality. Only associations can be identified between variables (Wang and Cheng, 2020).

For a more comprehensive understanding of causal relationships and the dynamic progression of DNA methylation patterns over time, a longitudinal study design would be more suitable. Such an approach could provide insights into the temporal changes of epigenetics and their potential role in disease development (Ng et al., 2012). While longitudinal designs could reveal these dynamics, establishing causality would require the longitudinal follow-up of initially healthy individuals to determine whether epigenetic changes precede the onset of binge eating or arise as a downstream effect. Nevertheless, the strengths of a cross-sectional study design were the reason for choosing this approach for this initial project.

Peripheral blood samples were collected and analysed in this thesis. This approach offers practical advantages, including ease of collection, minimal invasiveness, and straightforward storage requirements. Previous studies indicate that for some genes, DNA methylation patterns are largely consistent across different tissues (Reiner et al., 2023, Rönn et al., 2015), whereas for others,

tissue-specific differences are observed (Ma et al., 2014, Zhang et al., 2013). NOP is highly expressed in the brain. Therefore DNA methylation patterns in peripheral blood may not fully reflect those in brain tissue, as some studies report constant in methylation (Mendonça et al., 2024), while others highlight differences (Walton et al., 2016). To date, no study has specifically investigated whether NOP exhibits a tissue-specific DNA methylation pattern.

Taken together this highlights the difficulties involved in understanding the role of epigenetics in BED. Epigenetic modifications may not necessarily present as easily detectable differences in DNA methylation between groups at a single gene. Instead, such changes may be multilocalized, tissue-specific, and influenced by external factors such as environmental stressors or comorbidities.

4.6 Perspectives for future studies

Further research is necessary to deepen our understanding of the epigenetic mechanisms that might underlie BED. To that purpose, future studies should aim to recruit larger, more sex balanced cohorts ensuring comparable representation of sexes not only within BED and BED- groups but also across the overall study sample, therefore potentially improve the generalizability of the results as well to determine whether the observed sex-specific effects can be reproduced.

In addition, the inclusion of a healthy normal-weight control group would facilitate a more precise evaluation of the specific epigenetic changes associated with BED, thereby helping to distinguish between overall epigenetic alterations and those directly linked to the disorder. This design further enables researchers to disentangle DNA methylation patterns related to weight per se, which are present in both BED and BMI-matched obese controls but absent in normal-weight controls, from those uniquely associated with BED, which are present in BED but absent in both obese and normal-weight controls, as well as to identify more general epigenetic alterations that may occur across weight categories.

A variety of factors can influence DNA methylation levels, including medication, smoking, diet and nutritional intake, socioeconomic status, physical activity, stress, sleep patterns, and other environmental exposures (Amenyah et al., 2020a; Lee and Pausova, 2013; McGarel et al., 2015; Steiger and Booij, 2020; Tehranifar et al., 2013; Toth, 2021; Zhou et al., 2021). Given the large number and diversity of potential confounders, fully controlling for all of them is going to be challenging. To strengthen the robustness and generalisability of the findings, future studies should carefully consider these variables that may affect DNA methylation levels. The most impactful confounders should be prioritised, and insights from emerging research should be incorporated to identify additional factors that could influence epigenetic patterns.

Methodologically, expanding beyond a single-gene approach toward genome-wide analyses, as well as integrating additional epigenetic layers such as histone modifications and non-coding RNA regulation, would likely provide a more comprehensive understanding of the role, dynamic and mechanism of epigenetics in BED.

Genotyping should be employed in order to account for its potential influence on DNA methylation variability, as well as to identify potential genetic variants that may be linked with BED.

A longitudinal approach, in which DNA methylation patterns are tracked over time, would also provide valuable insights into their dynamics with regard to the onset and progression of BED.

From a diagnostic perspective, combining validated self-report measures with structured clinical interviews could improve diagnostic accuracy, and future work may benefit from incorporating measures of impulsivity, given its relevance to BED.

Finally, given the limited effects observed in NOP gene in this study, it is possible that other genes play a more prominent role in the epigenetic regulation of BED. Consequently, future research should explore additional candidate genes to better understand the genetic and epigenetic factors contributing to this disorder.

4.7 Outlook: DNA methylation in clinical practice

A growing understanding of the role of DNA methylation in disease has paved the way for potential clinical applications. The science of DNA methylation is most advanced in cancer research. As early as 1967, clinical trials began using DNA-methyltransferase inhibitors to specifically target DNA methylation in leukaemia (Ganesan et al., 2019). These inhibitors act on the enzyme DNA methyltransferase to prevent the methylation of cytosine residues. As a result, genes that were previously silenced, can be reactivated (Brueckner et al., 2005).

Initially, it was believed that high doses of inhibitors were necessary, based on the assumption that they would act similarly to classic chemotherapeutic drugs. However, such high doses caused significant side effects and were less effective than lower-dose regimens. The inhibitors are cytotoxic, with bone marrow suppression being one of the most severe potential side effects (Derissen et al., 2013).

Since 2008, the European Medicines Agency has approved DNA-methyltransferase inhibitors for the treatment of myelodysplastic syndromes, acute myeloid leukemia, and chronic myelomonocytic leukemia (Derissen et al., 2013).

Additionally it was also found that these drugs sensitise cancer cells to other forms of treatment, producing synergistic responses and reversal of drug resistance (Da Costa et al., 2017). They can also be used in combination with other medications that utilise different epigenetic effects, such as histone modification (Huang et al., 2024).

DNA methyltransferase inhibitors are primarily used for treating cancer and are not yet standard practice in other illnesses. This is partly due to the biological characteristics of cancer, which make it particularly susceptible to their effects. Many cancers are characterised by the hypermethylation of tumour suppressor gene promoters, which leads to unchecked cell growth. It's important to note that the inhibitors require newly synthesised DNA to incorporation into. This leads to a greater effect in cancer cells due to their rapid cell division (Gnyszka

et al., 2013). Although severe side effects occur, the risk-benefit profile is justifiable in life-threatening disease like cancers. By contrast for other illnesses, safer treatment options are established and might therefore be prioritised (Best and Carey, 2010). Furthermore, many non-cancer diseases might involve more complex or less well-understood epigenetic changes, as well as slower cell turnover (Mau and Yung, 2014). Therefore, it is harder to identify and manipulate targeted, disease-specific patterns without causing adverse effects in otherwise healthy cells, since the inhibitors are not gene- or locus-specific and affect DNA methylation throughout the whole genome (Gray and De Meyts, 2005, Suraweera et al., 2025).

Substantial preclinical research is necessary to understand fundamental concepts in epigenetics. One issue that should be addressed in this regard is the reliance on animal models, which provide experimental control and access to tissues that cannot be ethically or practically gained in human studies. Findings from these models may therefore not be fully translatable to humans. Additionally, some animal models undergo genetic manipulation as well as conditioning training to better fit the model. This may result in the disease being mimicked and only partially reflecting the truth about the condition (Best and Carey, 2010). It should be noted that many of the foundational studies on which this thesis is based were conducted using rodent models.

Besides treatment, DNA methylation could be used as a biomarker in clinical practice. This could be practical since changes can be detected in fluids such as blood, saliva, stool, or urine, which could be obtained in a form of a liquid biopsy as less-invasive sampling alternative to traditional tissue biopsies. However, to make this efficient, the sensitivity and reproducibility need to be improved so that it can be a reliable marker (Younesian et al., 2024).

Although risk assessment using DNA methylation as biomarker is not yet standard practice in mental illness, initial studies have begun exploring its potential. For example, one study suggested a methylation-based risk score could serve as a marker of resilience (Lu et al., 2022). Current research efforts

are investigating its applicability in mental disorders such as schizophrenia, bipolar disorder, major depressive disorder (Shirvani-Farsani et al., 2021), social anxiety disorder (Kwon et al., 2025) and in context with trauma/ Post traumatic stress disorder (Cao-Lei et al., 2022; Martin et al., 2021; van den Oord et al., 2022). Several candidate genes are currently under discussion in the context of eating disorders, with the majority of research focusing specifically on AN (Käver et al., 2024). Nonetheless, this remains a very new field of study.

In line with this, single-gene analyses like this thesis could help identify a biomarker and subsequently facilitate the development of future targeted therapies, as focusing on one gene is more practical for both measurement and intervention.

For mental health disorders specifically, there is a hypothesis that the focus should shift towards other epigenetic mechanisms beyond DNA methylation. Since DNA methylation is a more stable, less dynamic process, histone modification could be a more suitable approach, as many gene responses are initially stabilised via histone modifications before more permanent DNA methylation changes are established. At the same time, this higher level of dynamism can make histone modifications more difficult to specifically target or modulate. Therefore, it could be hypothesised that histone modifications might be useful in preventing damage in acute situations that could later contribute to mental health disorders such as post-traumatic stress disorder (Best and Carey, 2010). For BED, such interventions might be less promising. The disorder does not have a clearly defined point of onset, making it difficult to identify a specific window in which histone-based interventions could be applied.

If the development of individualised epigenomic profiling becomes feasible in the future, there is a chance that it could be used in the field of personalised medicine for assessing individual risk, as a marker of progress and for personalised treatment strategies (Mau and Yung, 2014). In such a scenario, large amounts of sensitive data would need to be managed responsibly to

prevent unauthorized access or exploitation, which could lead to ethical, social, and legal challenges (Santaló and Berdasco, 2022).

In summary, research in the field of epigenetics, and in particular DNA methylation, remains in its early stages. Although DNA methylation is the most extensively studied epigenetic mechanism, there is still much to be understood about how it can be used in practical applications. Nevertheless, further studies could create new opportunities, particularly in the development of therapies, risk assessment, diagnostics and personalised medicine.

5 Conclusion

This study highlights the complex relationship between epigenetics and BED, emphasizing the need for further investigation.

No significant difference in DNA methylation levels between BED+ and BED- individuals for NOP was found. A significant interaction between BED status and sex on DNA methylation levels was detected. In males, DNA methylation levels significantly differed between BED+ and BED- groups, specifically at CpG1. This finding aligns with previous research on sex-specific DNA methylation. But given the cohort-driven approach of the study further replication in diverse populations is necessary. This study found no significant correlation between BMI and DNA methylation levels across CpG sites. An exploratory approach revealed that high EDE-Q and restraint/shape concern scores might be linked to increased CpG methylation in individuals without BED.

In view of the modest effects observed for NOP in this study, it is possible that other genes may exert a more considerable influence on the epigenetic regulation of BED.

Further research is required to clarify the role of epigenetic mechanisms in BED, as the current body of knowledge on the subject is very limited. Consequently, future studies should include larger, sex-balanced cohorts and a

normal-weight control group. Research should be conducted with confounders included, employing genome-wide and multi-layered epigenetic approaches and integrating genotyping to capture genetic influences on DNA methylation. To elucidate the epigenetic mechanisms in BED, it is also necessary to use longitudinal designs and explore additional candidate genes.

6 Deutsche Zusammenfassung/ summary in German Language

In dieser Arbeit werden DNA Methylierungsgrade des NOP-Rezeptor-Gens im Zusammenhang mit Adipositas und Binge-Eating-Störung (BED) untersucht mit dem Ziel, potenzielle Unterschiede zwischen den Gruppen zu identifizieren.

Charakteristisch für BED sind wiederkehrende Essanfälle ohne anschließende kompensatorische Maßnahmen. Zudem tritt die Störung häufig gemeinsam mit anderen psychischen Erkrankungen auf (Mars et al., 2025). Trotz vielseitiger verfügbarer Therapieoptionen bleiben Remissionsraten oft unzureichend (Solmi et al., 2024).

Die Epigenetik befasst sich mit der Untersuchung Veränderungen der Genaktivität oder -funktion, die nicht auf einer Veränderung der DNA-Sequenz selbst beruhen (Dupont et al., 2009). Im Fokus dieser Arbeit steht die DNA-Methylierung.

Das NOP-Rezeptorsystem ist an der Regulierung des Fressverhaltens und der Energiehomöostase beteiligt. In Tier- und Humanstudien gibt es Hinweise auf eine Beteiligung des Gens an Binge Eating und Adipositas (Micioni Di Bonaventura et al., 2019).

In dieser Arbeit wurden 229 Teilnehmende (130 mit BED, 99 ohne BED) untersucht. Die Gruppen sind vergleichbar in Alter und BMI. Mittels Pyrosequenzierung wurden drei CpG-Stellen im ersten Intron des NOP-Rezeptors auf ihr DNA Methylierungsniveau hin analysiert. Es ergaben sich keine generellen Unterschiede zwischen den Gruppen, es wurde jedoch eine

Interaktion von Geschlecht und BED-Status gefunden. Männer mit BED zeigten höhere DNA Methylierung an einer der drei CpG Stellen. Es fanden sich keine BMI-Korrelationen mit DNA Methylierungswerten.

Die Ergebnisse verdeutlichen die Vielschichtigkeit epigenetischer Regulation bei BED, mit möglichen geschlechts- und ortsspezifischen Effekten.

Stärken der Studie sind die klinisch gut charakterisierte Stichprobe und standardisierte Methoden; Einschränkungen betreffen u. a. die geringe Zahl männlicher Teilnehmer, die Analyse peripherer DNA und das Querschnittsdesign.

Zukünftige Forschung sollte größere, geschlechtsausgewogenere Kohorten, weitere Kontrollgruppen, genomweite Ansätze und weitere Kandidaten Gene einbeziehen.

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8 Erklärung zum Eigenanteil

Die Arbeit wurde der Klinik für Psychosomatische Medizin und Psychotherapie (Innere Medizin VI) unter Betreuung von Prof. Dr. Katrin Giel durchgeführt.

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Ich versichere, dass ich sämtliche von den verwendeten KI-Tools gelieferten Hinweise kritisch geprüft, eigenständig bewertet und gegebenenfalls angepasst habe, bevor Inhalte in die Arbeit aufgenommen wurden.

Ich versichere, das Manuskript selbständig unter nach Anleitung durch Prof. Dr. Katrin Giel verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Tübingen, den

Unterschrift

Nicole Neuser

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10 Supplemental Data

This is the Data the Boxplot (see Figure 6) is based on.

Table to Panel A

DNA methylation Level [%] for Mean CpG

BED Status	Mean	SD	Minimum	Maximum	Median	Q25	Q75	Gender
BED-	95.3	1.43	90.1	97.9	95.5	94.6	94.6	-
BED+	95.3	1.76	86.7	99.7	95.7	94.7	96.5	-
BED-	95.1	1.8	90.1	97.2	95.5	94.4	96.6	m
BED-	95.4	1.3	90.8	97.9	95.5	94.6	96.3	f
BED+	96	0.91	94.6	97.7	96	95.3	96.6	m
BED+	95.2	1.85	86.7	99.7	95.6	94.3	96.4	f

Table to Panel B

DNA methylation Level [%] for CpG 1

BED Status	Mean	SD	Minimum	Maximum	Median	Q25	Q75	Gender
BED-	98.1	1.93	90.9	100	98.7	97.2	99.5	-
BED+	98.3	1.45	92.9	100	98.5	97.6	99.4	-
BED-	97.7	1.87	93.6	100	98.1	96.2	99.4	m
BED-	98.2	1.94	90.9	100	98.8	97.6	99.5	f
BED+	98.9	1.10	96.4	100	99.4	98.3	99.7	m
BED+	98.2	1.49	92.9	100	98.4	97.5	99.3	f

Table to Panel C

DNA methylation Level [%] for CpG 2

BED Status	Mean	SD	Minimum	Maximum	Median	Q25	Q75	Gender
BED-	90.7	2.1	80.4	94.7	91.1	89.7	92	-
BED+	90.2	3.59	71.3	94.3	91.2	89.4	92.3	-
BED-	90.4	2.92	80.4	93.8	91	88.7	92.4	m
BED-	90.8	1.78	85.3	94.7	91.1	89.8	92	f
BED+	91.3	1.71	88.4	94.2	91.7	90.1	92.5	m
BED+	90.1	3.8	71.3	94.2	91.2	89.4	92.3	f

Table to Panel D

DNA methylation Level [%] for CpG 3

BED Status	Mean	SD	Minimum	Maximum	Median	Q25	Q75	Gender
BED-	97.2	2.05	91.1	100	97.7	95.7	98.9	-
BED+	97.2	2.25	88.3	100	97.9	95.8	99	-
BED-	97.3	2.06	94.1	100	97.7	95.4	99.1	m
BED-	97.2	2.06	91.1	100	97.7	96	98.8	f
BED+	97.5	1.72	94.9	100	97.8	96.1	98.9	m
BED+	97.1	2.33	88.3	100	97.9	95.6	99	f